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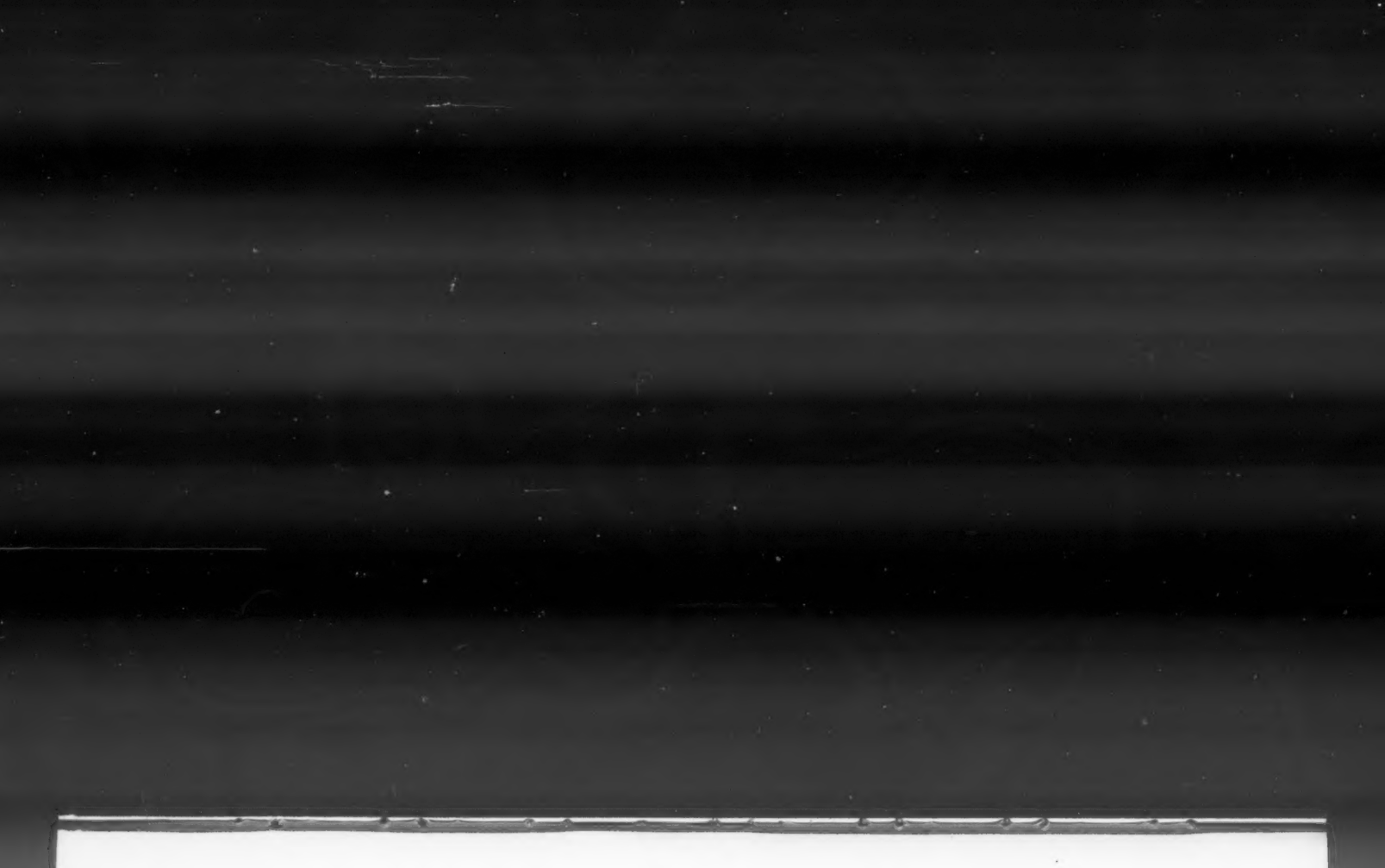

THE BOTANICAL REVIEW

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THE STRUCTURE AND BEHAVIOR OF FUNGUS NUCLEI

LINDSAY S. OLIVE

Department of Botany, Columbia University, New York City

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INTRODUCTION

This paper contains a discussion primarily of those investigations of nuclear cytology in the fungi which have appeared during the past 25 years. There are a number of mycology texts and review

papers which cover the more important cytological work prior to this period, and in the present review earlier papers will only occasionally be referred to for purposes of clarity and continuity. In general, the cytology of the cytoplasm with its extranuclear bodies will not be discussed. Also, no attempt is made to cover the field of fungus genetics, although certain genetic investigations will be referred to whenever they are considered particularly pertinent to an understanding of the cytological behavior. For an up-to-date discussion of the genetics of microorganisms, the reader is referred to the review paper of Tatum and Perkins (1950). Undoubtedly a number of noteworthy papers have been overlooked, but the writer hopes that the overall effect of this review will be to enable the reader to obtain a fuller understanding of the structure of the fungus nucleus and of its behavior throughout the life cycle of the organism. The reader will also undoubtedly wish to consult the reviews of Tischler (1927, 1931, 1936, 1938, 1942–1951), which contain discussions of cytological investigations in the fungi prior to 1942, and the recent more concise review of this subject by Cutter (1951).

The writer (1948), in a brief report, has outlined what he considers to be the main types of nuclear cycles in the fungi. Perhaps a short discussion of this subject here, including definitions of the more common terminology used in this paper, would be appropriate.

In most respects the fungus nucleus does not differ in structure or in behavior during division from the nuclei of higher plants and animals. There are present a nuclear membrane, chromatin strands, a single nucleolus and karyolymph. The nucleus divides by typical mitosis, during which a set of chromosomes of definite number and distinctive morphology may be discerned. A clearly defined equatorial plate arrangement of chromosomes during mitosis and meiosis appears to be much more characteristic of the phycomycetes and other lower forms than of the higher fungi. In general, the vegetative nuclei are quite small and difficult to study, and the greater part of our more accurate information on the nucleus has been derived from a study of meiosis, during which the nucleus is much larger and chromosomal details are much clearer than in the vegetative stage. The course of meiosis is very much like that in higher organisms. However, the spindle

appears to be almost exclusively intranuclear in origin. A study of nuclear structure in the vegetative phase of certain ascomycetes and basidiomycetes was made by Pinto-Lopes (1949).

One of the most outstanding features of the fungi—and one which has made them of particular interest to both cytologists and geneticists—is the variety of nuclear associations that exist among them. Some of these associations are not duplicated in any other organisms. In the great majority of fungi the nucleus exists in the haploid condition throughout the entire life cycle, up to the time the zygote is produced. Nuclear fusion occurs in the zygote, and the zygote represents the only true diploid stage in the life cycle. When its nucleus divides it undergoes meiosis. However, in a large number of these fungi (most ascomycetes and basidiomycetes) there is at some stage in the life cycle a unique association of the nuclei in pairs which divide conjugately. Upon reaching the cells destined to become zygotes, the pairs fuse. In many fungi (most phycomycetes) the haploid nuclei are not associated in pairs until the period immediately preceding karyogamy. Such fungi are referred to as existing primarily in the haplophase, the zygote being the only diploid cell. Fungi having the foregoing association of nuclei in the earlier part of the life cycle, in addition to a stage in which the nuclei are arranged in conjugate pairs, are said to exist both in the haplophase and in the dikaryophase, with the only diploid cell being the zygote. There is still a third type of life cycle (in *Allomyces* and some yeasts), apparently of uncommon occurrence, in which the organism has a prominent phase, known as the diplophase, in which the nuclei are diploid. This alternates with a prominent or reduced haplophase.

Some investigators have persisted in referring to the dikaryophase as a diplophase. This only leads to great confusion, particularly in view of the existence of true, well-developed diploid phases in the life cycles of certain fungi. The difference between the two terms is obvious. In the dikaryophase the haploid nuclei are associated in pairs; in the diplophase the two haploid sets of chromosomes are within a single nucleus. Experiments have shown that the components of a dikaryon may frequently be separated into different hyphae and later recombined. This is not possible with a diploid nucleus. Furthermore, dikaryons and diploid nuclei occur in different places in the life cycle of a fungus, and only diploid

nuclei undergo meiosis, not dikaryons. The writer believes that an effort should be made to emphasize the uniqueness of the dikaryophase to the fungi rather than to attempt to homologize it with the diplophase.

A discussion of homothallism and heterothallism becomes increasingly difficult in the light of recent investigations. It has been necessary to modify these terms as they were originally conceived by Blakeslee. It has generally been concluded from Blakeslee's work that a homothallic fungus is one which is able to complete its life cycle when grown from a single haploid spore. On the other hand, a fungus which requires pairing of compatible single-spore mycelia for the completion of the life history is said to be heterothallic.

Whitehouse (1949b) has presented what seems to be the most acceptable interpretation of homothallism and heterothallism which has appeared since Blakeslee's original discoveries. His essential definitions are the same as those of Blakeslee and as given in this paper. However, he has distinguished between the type of heterothallism involving morphologically distinct male and female thalli (as reported in a small number of fungi) and that involving a differentiation into strains showing no differences in sexual morphology (most heterothallic fungi). The former type has been referred to by Whitehouse as "morphological heterothallism", and the latter as "physiological heterothallism". Physiological heterothallism is further differentiated into bipolar and tetrapolar types, a single allelomorphic pair of factors controlling heterothallism in the bipolar type and two pairs controlling it in the tetrapolar type. The bipolar type is found to characterize heterothallic fungi in the phycomycetes, ascomycetes, rusts and smuts; whereas bipolar and tetrapolar heterothallism, with multiple allelomorphic factors occurring at the loci, appears to be characteristic of the Tremellales, hymenomycetes and gasteromycetes. In the few cases of morphological heterothallism which have been reported in the literature, the present reviewer has failed to find conclusive evidence that the sexual nature of the thalli can be determined when the thalli are maintained separately. Apparently distinctive sex organs are produced only when compatible cultures are brought together and there can be a *physiological* interaction between them.

Also, little is known concerning the genetics of sexual reproduction in such forms.

Several investigators have now demonstrated that quite a number of fungi can not be classified as either strictly homothallic or truly heterothallic. Sterility factors and possibly other genetic factors may alter the picture considerably. Some of these interesting intermediate types of fungi will be discussed in the main text of this paper.

The term " heterokaryotic " refers to a mycelium or any part of a fungus which contains haploid nuclei of more than one genotype. It is used to refer to a mixture of nuclei which differ in compatibility factors or in any other genetic factors. This term might also conceivably be used in reference to mixtures of haploid and diploid nuclei—a condition reported only rarely in the fungi—but it is generally not considered in this sense. The term " miktohaplont ", coined by Kniep, is probably best used in a reference to a fungus whose spores and mycelium regularly contain a mixture of nuclei of compatible mating-types. If the haploid nuclei in a mycelium or portion of a fungus are all genetically alike, the condition is described as " homokaryotic ". The terms " heterozygous " and " homozygous " are deemed applicable in the fungi as in other organisms only in reference to diploid nuclei. The same objections which have been raised in connection with the tendency of certain investigators to confuse the terms " dikaryophase " and " diplophase " apply here also. The bringing together of two different strains of a fungus to form a heterokaryotic mycelium is not referred to as " hybridization " but as the initiation of the dikaryon, or " dikaryotization ". The term " hybridization " is used as in higher organisms in reference to the fusion of genetically different nuclei. Dodge (1939*a*, 1942*a*, 1942*b*), who has discussed these matters more fully, coined the term " heterokaryotic vigor " in lieu of " hybrid vigor " for the increase in growth that sometimes occurs when a heterokaryon is initiated between two haploid mycelia. Dodge's usage of the term " mating types " is considered synonymous with the term " compatibility types " in this paper.

Probably the most logical approach to a discussion of such a large group of organisms is a phylogenetic one. Therefore, the fungi

to be considered are treated under their various taxonomic groupings arranged more or less in the conventional phylogenetic order.

MYXOMYCETES AND RELATED ORGANISMS

Some students of the fungi, with good reason, do not consider these organisms as true fungi and would prefer to relegate them to a position in the animal kingdom or to an intermediate position between plants and animals. However, since they have been all but ignored by others than mycologists, they will be included in this discussion.

The Plasmodiophorales are included here along with the slime molds, although it is by no means certain that the two groups are closely related. Even though both have multinucleate plasmodia and zoospores that are in some ways similar, they have little else in common. The Plasmodiophorales, unlike the myxomycetes, are obligate parasites and never form elaborate fructifications. There is also a method of nuclear division characteristic of this group which further distinguishes it from the myxomycetes and the true fungi.

MYXOMYCETES. It is now generally acknowledged that the plasmodium of the slime molds is initiated by the fusion of motile gametes and that the plasmodium, after feeding and enlarging for a time, gives rise to sporangia and spores characteristic of the particular species. Skupienski (1927, 1928) has claimed that in *Didymium difforme* the spores from the sporangium are of two compatibility types and that the one or two zoospores arising from each sporangiospore are of one compatibility type. In his opinion, therefore, it is necessary that gametes of opposite compatibility types be brought together before the plasmodium will be initiated. Such a species would be heterothallic, if the terminology may be extended to include such organisms. Cayley (1929), who studied the same species, reported that she obtained fructifications from single-spore cultures. Schünemann (1930) claimed that *D. difforme, D. nigripes, D. squamulosum* and *Physarum leucopus* are all homothallic. He watched the development of single zoospores into plasmodia and sporangia. However, von Stosch (1935) studied two forms or varieties of *D. nigripes* and found that one, var. *eunigripes*, is heterothallic, and that the other var. *xanthopus*, is homothallic. Data from pairing of single spore cultures of var.

eunigripes indicate that a single pair of compatibility factors is involved. Jahn (1936) has expressed the opinion that most myxomycetes are homothallic.

According to Skupienski, two or more gametes may fuse to initiate the plasmodium, and several plasmodia may also coalesce. He claimed that karyogamy does not occur just after plasmogamy, but that the haploid nuclei, with a chromosome number of 2, divide mitotically during growth of the plasmodium and fuse in pairs as the time of fruiting approaches. Several later investigators did not concur in this viewpoint. Wilson and Cadman (1928), in a cytological study of *Reticularia lycoperdon*, and Gilbert (1935), working on *Ceratiomyxa fructiculosa*, have reported that karyogamy occurs immediately after plasmogamy and that the plasmodium contains diploid nuclei throughout its development. Although many investigators have found that multiple fusion of gametes occurs in the slime molds, they are not all agreed as to the subsequent fate of the nuclei. Wilson and Cadman (1928) found that, in the case of multiple fusion of gametes in *Reticularia lycoperdon*, when two gametes fuse karyogamy occurs, and fusion of the resultant zygote with other gametes results in disintegration of the supernumary haploid nuclei. The latter reportedly divide frequently in an amitotic fashion before degenerating. The paper of Wilson and Cadman contains a useful review of the cytological work on the slime molds prior to 1928. Schünemann (1930) reported that multiple fusions of amoeboid zoospores and young plasmodia occur in *Didymium nigripes*, but that karyogamy is delayed and does not occur until later in the plasmodium. However, he did not actually observe nuclear fusion. Gilbert (1935) stated that in *Ceratiomyxa* multiple fusion of zoospores is followed by several nuclear fusions, whereas Jahn (1936) found that the accessory fusion of gametes with a young zygote results in the production of a plasmodium in which both diploid and haploid nuclei are mixed and may be found dividing mitotically.

As the plasmodium develops, the nuclei divide by typical mitosis, during which an intranuclear spindle appears and the nucleolus degenerates. The chromosomes, which are quite small, appear in an equatorial plate stage at metaphase, divide and pass to opposite spindle poles in two equal groups. Wilson and Cadman observed that 8 chromosomes, the diploid number, pass to each pole in *Retic-*

ularia, and Jahn (1933) found a diploid chromosome number of 16 in *Badhamia utricularis*. In the various species the size of the plasmodial nuclei appears to range between 2.5 μ and 6 μ in diameter.

Most recent investigators have agreed that meiosis occurs late in the life cycle of a slime mold. Wilson and Cadman observed that in *Reticularia* the two successive nuclear divisions in the sporangium just before the spores are cut out are meiotic. The delicate chromatin strands condense into 4 (presumably bivalent) small U-shaped chromosomes which become arranged in an equatorial plate on the metaphase spindle. The nucleolus disappears. During anaphase of the first and second divisions 4 chromosomes pass to opposite spindle poles. Sporangiospores, each containing a haploid nucleus, are cut out. During both divisions the spindles are intranuclear and centrosomes are absent. The haploid chromosome number was again observed during nuclear division in the zoospore prior to its division. However, the report of the peculiar extranuclear origin of the spindle (with centrosomes) in the zoospore is questionable. Howard (1931, 1932) was able to find only one simultaneous mitosis in the sporangium of *Physarum polycephalum*. Although he was unable to make chromosome counts, he believed this division to be meiotic.

Schünemann (1930) reported that in *Didymium nigripes* 8 chromosomes pass to each pole during mitotic divisions in the amoeboid zoospores and 16 during mitosis in the older plasmodium. He found two successive nuclear divisions preceding spore formation, but gave a confusing picture of chromosomal behavior at this point. According to him, uninucleate spores are cut out, and no further nuclear division takes place in them. Von Stosch (1935), on the other hand, observed that a single synchronous nuclear division occurs prior to spore formation in *D. nigripes*, and he considered the division as purely mitotic. At least 16 chromosomes were believed to be present. In *D. nigripes* var. *eunigripes* two successive divisions, considered by von Stosch as meiotic, were reported in the maturing sporangiospore. One nucleus was thought to degenerate after each division, thus leaving a single haploid nucleus in the spore. The haploid chromosome number was believed to be 24 or 25. The nuclei in the spores of *D. nigripes* var. *xanthopus* reportedly do not divide mitotically but are pre-

disposed towards amitotic budding. The species was concluded to be apogamous, and fusions between zoospores and plasmodia were considered to be of a vegetative nature. Von Stosch reported that this variety is probably triploid, as it has about three times the chromosome number of var. *eunigripes*. Von Stosch (1937) also studied the two successive nuclear divisions that occur in the spores of *Comatricha nigra* and considered them to be meiotic divisions. He found a haploid chromosome number of about 30 in this species.

In *Badhamia utricularis*, Jahn (1933) reported that after the nuclei pass through the recognizable stages of meiotic prophase, 8 pairs of chromosomes appear at the equatorial plate of the metaphase spindle and that 8 univalents, most of which measure around 0.5 μ, pass to each pole. The spores were reportedly cut out around these nuclei which obviously could not have completed the meiotic process. Jahn has suggested that the second meiotic division occurs when the spore germinates. According to him, certain investigations by Dröge (which had not been published at the time and which apparently have still not appeared in print) on a series of genera show that two nuclear divisions occur in the spore at the time of germination and that several zoospores may be produced, or three nuclei may degenerate and only one swarm spore emerges. If Jahn and Dröge are right, investigations into the homothallic or heterothallic nature of these organisms should start with single zoospores rather than single sporangiospores.

In *Ceratiomyxa*, which is an exosporous species, Gilbert (1935) reported that the single synchronous division of nuclei which he found in the developing fructifications prior to formation of the sporogenous cells is non-meiotic. Each uninucleate sporogenous cell gives rise to a single spore, considered by Gilbert to be homologous with a sporangium, in which the nucleus generally divides twice before the spore is discharged. These two divisions were thought to be meiotic. During prophase of the first division, up to 12 or 14 chromosomes were found to arrange themselves in pairs at " synapsis ". However, at the stage which was considered to represent synapsis, the chromosomes are short and thick, and the stage may be a much later one. About 8 chromosomes were believed to pass toward each spindle pole during this as well as the second division, indicating a reduction from 16 to 8 chromo-

somes. Jahn (1936) disagreed with Gilbert's interpretation of the developments in *Ceratiomyxa*. He believed that the last nuclear division before formation of the sporogenous cells is meiotic. He found that the nuclei pass through the typical stages of meiotic prophase, that 8 pairs of chromosomes appear on an equatorial plate at metaphase, and that 8 chromosomes pass to each pole during anaphase. Jahn considered the two successive nuclear divisions in the spore as mitotic, although the first of these would presumably represent the second meiotic division.

A number of investigators have given particular attention to the cytology of the zoospores. Wilson and Cadman (1928) as well as Sinoto and Yuasa (1934) and Yuasa (1935) claimed that the blepharoplast, from which the flagellum (or flagella) arises, is intranuclear in origin and remains attached to the nuclear membrane by a thin strand, the rhizoplast. In some cases the blepharoplast is composed of two granules, and when the zoospores are biciliate, a flagellum is attached to each granule. Yuasa stated that, although the nucleus is Feulgen-positive, the nucleolus and blepharoplast are Feulgen-negative. It was his contention that the blepharoplast is budded out by the nucleolus.

If culture methods can be improved to the extent that these organisms may be readily grown from single or paired gametes to the sporangial stage, they should prove of great interest in genetic as well as further cytological investigations. In nature there appear to exist in some species variants with phenotypic differences that would lend themselves well to hybridization studies. Such studies are much needed before we can adequately settle the argument as to where meiosis occurs in the life cycle of the slime molds.

PLASMODIOPHORALES. The life cycles and cytology of these organisms have already been reviewed in an excellent monograph by Karling (1942*a*). Therefore, the present discussion of this group will not be extensive. The obligate parasitism of these organisms has been a distinct drawback to an adequate study of the life cycle, and considerable confusion exists concerning nuclear developments. A representative life cycle for the group would probably be, according to Cook and Schwarz (1930), that of *Plasmodiophora brassicae*. According to these investigators, a spore which has been liberated into the soil germinates and produces a zoospore which infects the root hair of its host. A small

plasmodium develops in the root hair which gives rise to several uninucleate gametes. The gametes then presumably fuse in pairs, and the zygotes give rise to the larger plasmodia in the main part of the root. Much of this is speculation, since the so-called gametes were seen only in pairs and were not actually observed in the process of plasmogamy. Cytological studies by these same investigators show that nuclear divisions during the development of the zoosporangia are typically mitotic in appearance. However, in the large plasmodium a special type of nuclear division, believed to be characteristic of the Plasmodiophorales in general, was described. This has been called " protomitosis " by Cook and Schwarz (1930), Ledingham (1939) and others. It has also been referred to generally as a " cruciform division ". According to most investigators, a chromatin ring appears on an intranuclear spindle and surrounds the nucleolus (karyosome). The latter becomes dumbbell-shaped while the chromatin ring, in which individual chromosomes may or may not be distinct, splits and a ring of chromatin passes to each pole. The nucleolus constricts into two parts, one of which passes into each daughter nucleus.

Cook and Schwarz believed that meiosis occurs in the last two divisions prior to spore formation. But just before this an intervening phase, called the " akaryote " stage, was described, during which the nucleolus breaks up. Following this most of the chromatic material in the nucleus is extruded into the cytoplasm, where it can be seen as densely stained granules. In earlier times some workers claimed that these granules reorganize into new nuclei, but this theory has been discredited. According to Cook and Schwarz, a small portion of residual chromatin remains in the nucleus, and this chromatin condenses into small chromosomes which become arranged in an equatorial plate on the intranuclear spindle and separate into two groups which pass toward their respective poles. The second-division spindles are similar but smaller. Although chromosomes were not counted, the divisions were considered meiotic. At the end of the second division uninucleate spores are cut out.

Milovidov (1931, 1933), using refined staining techniques, was able to demonstrate the presence of distinct chromosomes (although he was unable to count them) in the ring surrounding the nucleolus during somatic mitosis. He also observed the division of the

nucleolus. With the use of the Feulgen technique he was able to demonstrate that the nucleolus, unlike the chromosomes, gives no reaction for thymonucleic acid, thus adding evidence to the belief that it is homologous with the nucleolus of other organisms. It was also demonstrated that, during the so-called akaryote stage, the chromatin never completely disappears from the nucleus, as some investigators have reported. In the two nuclear divisions just prior to spore formation, stages resembling meiotic prophase were observed. Nuclear divisions similar to those observed by Cook for this stage were reported. Chromosome counts were not made. During both these divisions, as well as in somatic mitosis, spindles with centrosomes and astral rays were observed. Milovidov has suggested that here, as in the ascomycetes, the centrosome-astral ray mechanism may cut out the spores.

Cook (1928) found that in *Ligniera junci* the plasmodial nuclei measure about 2.5 μ. Somatic divisions were found to be protomitotic. During the akaryote stage the nucleus was said to become completely colorless in stained preparations because of the extrusion of the nucleolus and of all the chromatin into the cytoplasm where they take the stain. Chromatin was thought to reappear in the nuclei. During the first meiotic division a maximum of 16 chromosomes was estimated to be present as the diploid number. Typical mitotic divisions were found in the development of the zoosporangia. Similar nuclear divisions were reported by Cook (1931) in *Sorodiscus radicicolus* which possibly has a diploid chromosome number of 4. The plasmodium was described as originating from the fusion of two amoeboid gametes with an accompanying karyogamy. Wernham (1935), in a study of a species of *Sorodiscus* on *Heteranthera*, found that cruciform divisions occur during growth of the plasmodium and that the last two divisions preceding spore formation are probably meiotic. Spindles with astral rays, but without centrosomes, were reported. From 4 to 6 pairs of chromosomes were believed to appear on the metaphase plate in the first division.

In a study of *Spongospora*, *Sorosphaera* and *Plasmodiophora*, with most of the observations confined to *Spongospora*, Horne (1930) found that somatic nuclear divisions are mitotic and of the cruciform type. The resting nucleus contains a nuclear membrane, a conspicuous nucleolus and linin strands with granules or

rods of chromatin. Nuclear divisions in the growing plasmodium are of the typical cruciform type, with 4 chromosomes present. Chromatic particles are extruded from the nucleus during the transitional (akaryote) stage. Horne's contention that karyogamy occurs during this stage was not proven by the observations. Diploid nuclei with 4 pairs of chromosomes are supposedly produced, and 4 chromosomes pass to each pole in the ensuing division, which is typically mitotic (not cruciform) in appearance. However, following this division the nucleolus reappears in each daughter nucleus and the second or homotypic division is of the cruciform type. It was found that a third division may occur before spore formation. The most serious objections to Horne's contention that karyogamy occurs during the transitional phase are that nuclear fusion was not actually observed and that the much condensed appearance of the chromosomes at the time when synapsis was believed to occur indicates a misinterpretation of the material.

Webb (1935) studied the cytology of *Sorosphaera veronicae* (Figures 1–16) and found that cruciform divisions occur during plasmodial development. The chromatin ring was found to be composed of 4 distinct chromosomes. Webb has pointed out that, although the nucleolar material appears to be extruded into the cytoplasm during the transitional stage, the chromatin persists in the nucleus. Like Horne, he believed that nuclear fusion occurs at this stage, but he did not actually observe it. The nuclear division which follows was considered to be meiotic, and the characteristic stages of meiotic prophase were described, during which the nucleolus (which has reappeared) degenerates and centrosomes with asters appear. Four bivalent chromosomes become arranged in a ring on the spindle, and a ring of 4 chromosomes passes to each pole. In the second or homotypic division 4 univalents pass to each pole, after which the uninucleate spores are cut out. The spindle fiber attachments apparently occur near the centers of the chromosomes. It is obvious that, according to the concepts of Horne and Webb, the vegetative phase of the Plasmodiophorales is haploid; whereas, according to Cook and others, it is diploid. Cytological studies of a more critical nature are needed here. There is a good possibility that carmine or orcein squash preparations would be helpful.

Whiffen (1939) has reported that in *Octomyxa achlyae* nuclear

divisions in the zoosporangia are typically mitotic in appearance. Karyogamy was believed to occur during the transitional stage. Meiosis is reportedly accomplished by means of a quadripolar spindle which appears to be unique to this species. Couch et al. (1939) reported that the nuclear divisions in the plasmodium of this parasite are of the protomitotic type.

As Cook has previously pointed out, the cruciform type of somatic nuclear division is such a distinctive feature of the Plasmodiophorales that it is to be considered of diagnostic value, and it is doubtful that any organism lacking this type of division should be placed in the group. Most modern investigators of these organisms apparently agree.

PHYCOMYCETES

This group is considered by many to be the most primitive class of the true fungi. The thallus varies from a simple monocentric type with a single nucleus to a much-branched coenocytic mycelium with unlimited growth potentialities. In general, the vegetative thallus is believed to be haploid, with the diploid stage confined to the zygote. It should be pointed out that such a condition is frequently assumed rather than proven, since most of the cytological work on this subject has been inconclusive. One exceptional group in which an alternation of well-developed haploid and diploid generations has been demonstrated will be discussed presently. Most of the phycomycetes have been a rather unrewarding group for cytological study, since the nuclei are generally quite small and, with few exceptions, the fusion nuclei do not attain the size of the fusion nuclei of many ascomycetes and basidiomycetes. Asexual reproduction in the group is by means of uninucleate or multinucleate motile or non-motile spores, and sexual reproduction is accomplished in a variety of ways, including fusion in pairs of isogamous or heterogamous motile gametes, fusion of a small motile male gamete with a large non-motile female gamete, plasmogamy between multinucleate antheridium and oogonial eggs, and copulation of two multinucleate gametangia which results in the production of a multinucleate zygote. The phycomycetes, more than any other group of true fungi, are in need of more critical cytological and genetic studies to help solve the many problems which exist concerning karyogamy, meiosis and mating reactions.

CHYTRIDIALES. An excellent review and extensive bibliography on the life cycles and cytology of chytrids investigated prior to 1937 may be found in a paper by Karling (1937). Chromosome numbers for species in which counts have been made by previous investigators are listed in this paper. The amount of cytological research in the group during the past two decades has not been extensive. Schwarz and Cook (1928) investigated the life history and cytology of the holocarpic *Olpidium radicale* and found that uninucleate zoospores, upon infecting the host, give rise to uninucleate thalli. Thalli may develop into zoosporangia, or they may fuse in pairs to form zygotes, after which karyogamy occurs. Nuclear divisions following karyogamy were described, but chromosomes were not counted. The fusion nucleus during prophase extrudes chromatic granules into the cytoplasm, after which it reportedly divides meiotically. Although a few mitoses were described during the life cycle of this organism, the writers stated that they were not certain but that amitosis might also occur. Cook (1929, 1932) reported simultaneous mitotic divisions in the developing sporangium of *Cystochytrium radicale*, and, although a small number of chromosomes was found to appear, an accurate count could not be made. Hovasse (1936), who described the last two mitoses in the sporangium of *Rhizophydium beauchampi*, found that the nuclei divide more or less simultaneously. Intranuclear spindles with centrosomes and an undetermined number of small chromosomes were reported.

Undoubtedly, one of the most detailed accounts of the cytology of chytrids up to the present time is that of Kusano (1930) for *Synchytrium fulgens*. In the formation of the thin-walled sporangia or gametangia, the thallus of this obligate parasite at first contains a single nucleus with a few chromatin granules. The nucleolus reportedly arises spontaneously. As the thallus enlarges, the nucleus and nucleolus enlarge greatly. (The present writer, in an examination of slides of certain species of *Synchytrium* prepared by Dr. M. T. Cook, finds that the nucleus may reach a diameter of 80 μ, although the spindle of the first division is comparatively much smaller.) Simultaneously the chromatin granules and linin threads appear to increase in number. Later the nucleolus extrudes chromatic globules into the cytoplasm. At the end of the period of vegetative growth, discharge of the globules ceases

and the chromatin appears as an extensive group of strands in the nuclear cavity. All but a small portion of these strands degenerate. Discharge of globules from the nucleolus recommences as the chromatin strands condense into 5 small chromosomes on an intranuclear spindle. By this time the nucleus is greatly reduced in size. Both the nucleolus and nuclear membrane eventually degenerate and new membranes are formed around the daughter nuclei. Following this, the nucleolus of each daughter nucleus goes through a period of discharging globules into the cytoplasm, and the chromatin strands decrease in amount, the remaining strands again forming 5 chromosomes. Astral rays appear to assist in the delimitation of the nuclear membrane after each division. Further divisions occur, and multinucleate gametangia, in which still more nuclear divisions take place, are cut out. Uninucleate gametes are produced.

During germination of the thick-walled zygote, Kusano observed stages in the division of the zygote nucleus similar to those already described. However, 5 bivalent chromosomes appear here. Cytologists and geneticists alike will look with suspicion upon the report that the chromosomes divide transversely. The first division, believed to be heterotypic, is followed by a number of successive divisions which lead to the production of multinucleate gametangia, as already described.

The report by Kusano and others that part of the chromatin deteriorates just before the nucleus divides needs further study. There can be no doubt that the total volume of the chromosomes on the metaphase spindle is much less than the volume of chromatic material, exclusive of the nucleolus, in the metabolic nucleus. It is also obvious that chromatic material, other than that derived from the nucleolus, is discharged into the cytoplasm. Nevertheless, it is probably safe to conclude that this material is not euchromatin, but that it is derived from portions of the chromosomes not concerned with the heredity of the organism.

Köhler (1931) studied the life history of *S. endobioticum* on the white potato and found that motile uninucleate gametes from the thin-walled summer sporangia copulate in pairs, following which the two nuclei fuse. Several cases were observed in which three gametes fused, and, although two of the nuclei generally fuse and the third degenerates, a few instances were reported in which all three nuclei fused. Köhler found that both gametes and zygotes

may infect the potato. The former produce summer sori (thin-walled sporangia) and the latter produce resting sporangia. The two types of thalli were said to be cytologically distinguishable in early stages, since a thallus derived from a zygote has two " Randkörper ", while the thallus produced by a single gamete has only one. The " Randkörper ", which disappear two to three days after infection, apparently are the remains of blepharoplasts.

Only a few papers have appeared recently on the cytology of eucarpic chytrids. The evidence available shows that the nuclei in the thalli of these organisms are confined to the sporangia and resting sporangia and do not occur in the rhizoids or rhizomycelium, as the case may be. Hillegas (1940) has reported typical intranuclear mitosis in the sporangia of *Endochytrium operculatum*. He found cone-like centrosomes at the spindle poles with astral rays emanating from them. Short rod-like chromosomes of undetermined number were found to divide on the spindle. The resting sporangia, which are predominantly uninucleate, were believed to develop asexually. Similar mitotic divisions were observed by Hanson (1945) in *Rhizophydium coronum*, where the chromosome number (haploid) was estimated to be from 6 to 8. In *Catenochytrium* she (1946) observed that the chromosomes appear on the spindle in ring formation. The chromosome ring splits, and a ring of chromosomes passes to each spindle pole. This type of chromosomal behavior reminds one of the kind of nuclear division found in the vegetative phase of the Plasmodiophorales. Here, however, the nucleolus degenerates without dividing.

In *Cladochytrium replicatum*, a polycentric species, the nuclei in mature thalli, according to Karling (1937), are confined to the " spindle organs ", zoosporangia and resting spores. In both the spindle organs and the sporangia intranuclear spindles with distinct chromosomes appear. The latter were believed to number from 6 to 9. The chromosomes become arranged in a ringlike band at metaphase and split in two, one band of chromosomes going to each spindle pole. The nuclear membrane persists an unusually long time, not disappearing until telophase, after which the nucleolus becomes stranded in the cytoplasm where it degenerates.

BLASTOCLADIALES. This group is unique in being the only class of true phycomycetes known to contain species that show a distinct alternation of extensively developed sexual and asexual generations. This condition, unusual in the fungi but common among the algae,

was first demonstrated in *Allomyces* by Kniep (1929, 1939). The importance of these organisms in genetic and cytological research has been recently pointed out by Emerson and co-workers. According to Tischler (1938), Sörgel reported in 1936 a basic haploid chromosome count of 6 in *Allomyces kniepii*, but he also found polyploid mutants with 12, 15 and 18 chromosomes. Sörgel (1937) has made one of the most detailed studies of the life history of *Allomyces*, in which he described the numerous disturbances which may occur in the alternation of generations. He found that a gametophytic mycelium, probably by duplication of the chromosome number in one or more nuclei, could give rise to a sporophytic mycelium, and that a sporophytic mycelium, apparently through somatic reduction, could give rise to a gametophytic mycelium. Sörgel also found that macrogametes occasionally develop into sporophytic mycelia, thus demonstrating unreduced parthenogenesis, an apparently rare phenomenon in the fungi. His conclusions regarding nuclear developments were based in part upon actual measurements of nuclei, which showed that sporophytic nuclei average about twice the size of gametophytic nuclei, but he also claimed to have data which showed that the chromosome count in the sporophytic nucleus is twice that of the gametophytic nucleus. Beneke and Wilson (1950) have shown that treatment of germinating zygotes of *A. javanicus* var. *japonensis* with sodium nucleate, and possibly also with colchicine, increases the frequency with which the resting sporangia will produce zoospores that give rise to sporophytic thalli in addition to gametophytic thalli. The results were believed explicable on the basis of the induction of polyploidy during zygote germination. Whiffen (1951), in a most interesting study of the effect of cycloheximide (Acti-dione) on the sporophyte of *Allomyces arbuscula*, has confirmed some of Sörgel's observations. She observed that this antibiotic substance, when incorporated into the agar medium on which sporophytes of *Allomyces* are grown, stimulates the production of hyphal outgrowths bearing male and female gametangia. Such hyphal outgrowths, when transplanted, give rise to mycelia that are mixtures of sporophytic and gametophytic parts and which eventually become entirely sporophytic or gametophytic. It is suggested that cycloheximide may interfere with mitotic division in such a way as to cause separation of the chromosomes into two groups without their splitting. This may be similar to the " reductional group-

ings" described by Huskins, Wilson and others in higher plants. Whiffen's paper should stimulate further basic research on the genetic and cytological effects of antibiotics upon fungi.

Hatch (1935) was the first to study the nuclei of *Allomyces*. He found them to be typical in appearance, containing nucleolus, chromatin strands and nuclear membrane. In his study of gametogenesis in *A. arbuscula* he described spindles with about 6 chromosomes passing to each pole. In a later investigation (1938) he observed plasmogamy and karyogamy. Following the fusion of unequal gametes, the nuclear caps were found to fuse, but the nuclei delay fusion until zygote germination. Hatch stated that the chromosome number is reduced in the first meiotic division—at germination of the zygote, according to him—from 12 to 6, and that in the second meiotic division the 6 chromosomes split equationally, 6 chromosomes going to each daughter nucleus. Thus Hatch believed that both sporophytic and gametophytic generations are haploid. Emerson and Wilson (1949), in a study of chromosome numbers in *Allomyces* with aceto-orcein technique, found that meiosis occurs during germination of the resting sporangia. Their chromosome counts were later found to be incorrect and were emended by Emerson (1950) and Wilson (1952). The latter paper is a fine contribution to the cytology of the phycomycetes, and the photographs represent the clearest and most convincing figures of meiotic chromosomes published for this class of fungi. Wilson's paper fully substantiates Kniep's claim of alternation of haploid and diploid generations in *Allomyces* (Figures 26 and 27). The haploid number of chromosomes was found to be 16 in *A. arbuscula*, 28 in *A. javanicus* var. *macrogynus*, and 14 in *A. cystogenus*. It was shown that meiosis does not occur in *A. anomalus*, in which the resting sporangium, upon germination, gives rise to diploid zoospores that produce sporophytes. In *A. cystogenus* the sporophytic phase is well-developed, but the gametophytic stage is reduced to a single cell—the encysted spore derived directly from a zoospore produced by the germinating resting sporangium. This cell contains two haploid nuclei, each of which divides once mitotically—the only division of a haploid nucleus in the life cycle of the species—and four uninucleate isogametes are produced. These fuse in pairs and the resulting zygotes produce the sporophytic plants.

Wilson has also reported the occurrence of polyploidy in the

group. Haploid numbers of 8, 16, 22–24 and 32 have been found in various isolates of *A. arbuscula*, and in *A. javanicus* var. *macrogynus* up to 50 or more chromosomes have been reported in the haplophase of certain isolates. *A. javanicus* var. *javanicus* has been found to have from 13 to 21 chromosomes, and its sporophyte is considered to be a natural hybrid with variable chromosome number. Wilson also observed that the chromosomes are morphologically distinct and that the nucleolus chromosome is relatively large, with a long secondary constriction near one end.

Cotner (1930*a*) studied the zoospores of *Blastocladia pringsheimii* and *B. globosa* and observed that the single flagellum is attached, with an intervening blepharoplast, to the pointed proximal end of the nucleus. Hatch (1935, 1938) believed that what Cotner considered to be the dense proximal part of the nucleus is, in reality, a nuclear cap of chondriosomal origin. He found that the flagellum is attached to the nucleolus by means of a rhizoplast. The nucleolus was described as constantly moving back and forth with respect to the point of attachment of the flagellum to the plasma membrane, alternately relaxing and distending the nuclear membrane in its movement.

MONOBLEPHARIDALES. Very litle information is available on the nuclear cytology of this group. This is probably due mainly to the difficulty usually experienced in culturing these fungi in the laboratory. Laibach (1927) studied several members of the class cytologically and found that the nuclear developments were essentially the same in all. Most of his observations were made on *Monoblepharis macrandra*. The mycelium is coenocytic, containing nuclei with nucleolus, chromatin strands and nuclear membrane. The nuclei multiply in the terminal zoosporangia which are multinucleate at their origin, and uninucleate zoospores with nuclear caps are produced. The male gametangia develop in much the same way as the zoosporangia but contain fewer nuclei and produce fewer swimming cells (microgametes). The female gametangium is uninucleate from its origin, and no nuclear divisions occur within it, a single large non-flagellate macrogamete being produced. When a microgamete swims to a macrogamete and fuses with it, the two nuclei pair. The developing zygote then migrates out of the female gametangium in a somewhat amoeboid fashion and encysts at its mouth. As the wall thickens, nuclear fusion

occurs. Following a dormant period of several months, the resting spore germinates and the fusion nucleus undergoes several successive divisions before the germ tube appears. A synapsis-like stage appears during prophase of the first division, indicating that meiosis occurs at this point. Apparently, actual nuclear divisions were not observed here or elsewhere in the life cycle. Although these fungi appear to be fairly closely related to the Blastocladiales, there has thus far been no evidence that an alternation of distinct haploid and diploid generations occurs here.

SAPROLEGNIALES. The writer follows Karling (1942*b*) in placing the holocarpic biflagellate species in this order, along with the more complex forms. Karling's monograph includes a review of the cytological investigations of the holocarpic group. These investigations have not been extensive either before or since the publication of this monograph. McLarty (1941) found that, during development of the thalli of *Olpidiopsis achlyae*, the nuclei divide synchronously by means of intranuclear spindles with centrosomes. The nuclear membrane and nucleolus degenerate, and 6 chromosomes pass to each spindle pole. The suggestion by McLarty that the nucleolus acts as a storage body for chromatin and assists in the elaboration of the chromosomes would probably not stand up under more modern cytological techniques. Although plasmogamy between unequal multinucleate thalli occurs in *O. achlyae*, karyogamy and meiosis were not observed. McLarty demonstrated, with single-spore infections, that *O. achlyae* is homothallic.

The bulk of the cytological studies in the Saprolegniales has been confined to the Saprolegniaceae. The majority of investigators are in agreement on the salient features of the cytological developments (Figures 17–25, 28). It is generally conceded that both antheridium and oogonium arise from coenocytic hyphae as multinucleate branches, and that during their further development at least one more or less synchronous mitotic division occurs in both organs. The nuclei are similar in structure and in mode of division to those already described as typical for the phycomycetes. As the sex organs approach maturity, most of the nuclei in the oogonium degenerate. This is also accompanied by nuclear degeneration in the antheridium. The remaining oogonial nuclei (one to several) persist as the functional egg (oosphere) nuclei and a mass of protoplasm is delimited around each. Typically, the

antheridium produces fertilization tubes equal in number to the eggs in the oogonium, and each fertilization tube deposits a single nucleus in the egg, where it fuses with the egg nucleus. By far the majority of investigators are of the opinion that meiosis occurs upon germination of the zygote (oospore). It should be emphasized here that, as in most phycomycetes, the nuclei and nuclear spindles are quite small, and that most statements regarding chromosome numbers during nuclear divisions in the germinating zygote or elsewhere in the life cycle have been made with reservations. It should be further emphasized that most investigations of the cytology of these and other fungi have been made with stains, such as iron alum haematoxylin and crystal violet, which stain spindle fibers as well as chromosomes. In view of the fact that the chromosomes are so small and that the spindle fibers frequently become drawn together in several main strands against which the chromosomes may not be clearly distinguishable, it is not surprising that so much difficulty has been experienced in obtaining accurate chromosome counts. Since the carmine and orcein techniques, which are relatively simple to use, do not stain the spindle, the chromosomes may be observed much more clearly in the nuclear vacuole during division as well as in prophase, and the technique may prove useful in a study of the Saprolegniales. Ziegler (1953) recently used it in a study of several species of water molds, but he reports that his results with other stains were better. He observed what appear to be stages in meiotic prophase preceding nuclear division in the germinating zygotes.

Concerning the nuclear divisions in the sex organs, Mäckel (1928), in a study of *Saprolegnia thureti* and *S. mixta*, concluded that several nuclear divisions occur in the oogonium. He observed that the persistent nuclei, each with distinct centrosome and aster, become the centers of development of the eggs. He believed that the centrosome and astral rays play a major part in cutting out the egg cytoplasm. Shanor (1937), in his investigation of *Thraustotheca clavata* (Figures 17–25), also believed that astral rays play an important part in delimitation of the eggs and probably of the zoospores. Schrader (1938) also thought that asters assist in delimiting the zoospores of this species. Höhnk (1935) and many others have reported asters following mitosis in the oogonium, but have not attributed to them any particular function. Höhnk was

of the opinion that they did not assist in egg delimitation. According to Cooper (1929*a*, 1929*b*) and others, a radiating central vacuole is primarily responsible for cutting out the eggs.

The report by Dangeard (1930) of nucleolar fragmentation and a type of division resembling amitosis in *Saprolegnia* appears to have resulted from a study of poorly prepared material.

Couch (1932), in a developmental study of the sex organs of *Leptolegnia caudata*, has reported a development in the oogonium which somewhat resembles the type of development found in higher Saprolegniales and in lower members of the Peronosporales. Following mitotic division in the oogonium, one nucleus with centrosome and astral rays remains in the center of the single developing egg, while all the other nuclei migrate to the peripheral region and degenerate. The antheridium, instead of producing a fertilization tube, fuses directly with the oogonium and deposits its single persistent nucleus which fuses with the egg nucleus.

Karyogamy has been reported for various species by a number of investigators, but detailed descriptions of chromosomal behavior at this stage are not available. Mäckel (1928) observed that the oospores of *Saprolegnia thureti* in most cases develop apogamously, while in *S. mixta* they may develop sexually or apogamously. Schrader (1938) reported that the fusion nucleus of *Thraustotheca clavata*, at the time of zygote germination, undergoes two successive divisions which are probably meiotic. The fusion nucleus was found to be 4 to 4.5 μ in diameter. The first division, considered heterotypic, requires a considerably longer period for its completion than the second, or homotypic, division. Final evidence that these divisions are meiotic is lacking, as the chromosomes were not counted during the various phases of division.

Heterothallic behavior has been reported in a few of the Saprolegniaceae; therefore, genetic studies involving crosses between different strains of such species should aid greatly in definitely establishing where meiosis occurs. The best genetic evidence that meiosis occurs at zygote germination has been presented by Couch (1926) in a study of germinating oospores from crosses between compatible strains of *Dictyuchus monosporus*. This report contains the first demonstration of heterothallic behavior in a water mold. The investigations of Raper (see Bot. Rev. 18: 447–545. 1952.) on heterothallism and the hormonal mechanism in certain

species of *Achlya* further indicate that a genetic approach to the study of meiosis would be feasible and most interesting in such forms, although there have as yet been no reports of oospore germination in the species studied by Raper.

Most of the chromosome counts for the Saprolegniaceae have been determined from mitotic divisions in the sex organs, primarily in the oogonium. Since precise counts are rarely if ever possible, most of the figures given must be considered as estimates. The more recent reports are listed as follows: *Saprolegnia ferax*, 7 or more (Höhnk, 1935); *S. thureti*, *S. mitxa*, *S. monoica*, *Achlya debaryana* and *A. prolifera*, 11 (Mäckel, 1928); *A. racemosa*, 4–6 (Carlson, 1929); *A. conspicua*, 4 (Moreau and Moreau, 1935); *A. flagellata*, 4 (Moreau and Moreau, 1935; Wolf, 1938); *A. bisexualis*, 8 or more (Raper, 1936); *Thraustotheca clavata*, 11 (Shanor, 1937) and 5 (Schrader, 1938); *Brevilegnia diclina*, 4–8 (Cooper, 1929*a*); *Leptolegnia caudata*, 8 (Couch, 1932). In addition, Ziegler (1953) has recently reported the following chromosome numbers: *Saprolegnia litoralis*, 7; *Achlya colorata*, 3; *A. recurva*, 4; *A. megasperma*, 5; *Isoachlya intermedia*, 6; *Thraustotheca primoachlya*, 5. All of these are haploid numbers.

Recent cytological studies on the Leptomitaceae and the Rhipidiaceae have shown that certain stages in the ontogeny of the sex organs resemble the developments in certain of the Peronosporales more than those considered characteristic of the Saprolegniales. This is not surprising in view of the fact that the two orders appear in several other ways rather closely related phylogenetically. In *Apodachlya brachynema* and *Sapromyces reinschii* (Kevorkian, 1935) and in *Rhipidium europaeum* (Behrens, 1931) the contents of the oogonium differentiate into periplasm, ooplasm and coenocentrum. After mitotic division of the nuclei in the oogonium (and antheridium) one nucleus remains in the coenocentrum, while the others migrate into the periplasm and degenerate. Only one egg is produced. Fusion of antheridial and egg nuclei has been reported by Kevorkian who observed that meiosis probably occurs at germination of the zygote. Behrens found the two nuclei pairing in the developing zygote, but did not observe their fusion. He stated that karyogamy probably occurs prior to germination of the zygote and that reduction division follows. However, he did not actually observe stages in zygote germination.

The cytology of the zoospores of the Saprolegniales has been studied in some detail by several investigators. Matthews (1932) observed that, as the zoospore matures in the sporangium of *Leptolegnia caudata*, a central body in the nucleus—probably a nucleolus—pushes out a beak-like process which has a centrosome at its summit. The two flagella were believed to arise from the centrosome. Apparently he did not believe that a true blepharoplast is present. Cotner (1930*b*) studied the development of the zoospores in genera of the Blastocladiales, Saprolegniales and Peronosporales. He found that the so-called "basal apparatus" is of nuclear origin and appears to be composed of several chromatic bodies, including a distinct basal granule or blepharoplast to which the flagella are attached, a rhizoplast connecting the blepharoplast with the nucleus, and several granules which are retained in the nucleus where they form the beak-like part of the nucleus during zoospore activity. Cotner believed that when a zoospore comes to rest, the basal granule or granules migrate back into the nucleus and again become a part of it.

PERONOSPORALES. The nuclear cytology of this group is in general similar to that described for the higher Saprolegniales, with a few notable exceptions. One or more generally simultaneous nuclear divisions in oogonium and antheridium, differentiation of the oogonium into periplasm and ooplasm, and fertilization of a single uninucleate egg are described for most species. At the completion of mitosis in the oogonium, a single nucleus persists as the functional egg nucleus, while the remaining nuclei migrate into the periplasm and degenerate. The egg nucleus is frequently surrounded by a small densely staining sphere of cytoplasm called the "coenocentrum". Likewise, a single antheridial nucleus persists and is carried into the egg cytoplasm by a fertilization tube. In the great majority of species the nuclei are reported to be of the conventional type and to divide by typical intranuclear mitosis. Sporangia are multinucleate at their origin, and the zoospores, in those species which produce them, are uninucleate.

The developments described above are particularly characteristic of the Pythiaceae and Peronosporaceae. In *Phytophthora cambivora* (Allain, 1934), in *Peronospora effusa* (Tsang, 1929) and in *Sclerospora* (McDonongh, 1937, 1946) two successive mitotic divisions have been described in the sex organs, at least in the

oogonium, while in *Phytophthora cactorum* (Blackwell, 1943) two or three nuclear divisions take place in the young sex organs before they are delimited, and a simultaneous mitosis in both organs occurs as they approach maturity. Mundkur (1949) has reported that several successive nuclear divisions appear to occur during development of the sex organs of *P. himalayensis*. Developments before and after karyogamy are varied. Mundkur has stated that, following nuclear degeneration in the oogonium of *P. himalayensis*, the single persistent oogonial nucleus divides, one daughter nucleus degenerating and the other becoming the functional egg nucleus. Although most investigators have found that karyogamy follows shortly after plasmogamy, Arens (1929) found that in *Rhysotheca* (*Plasmopara*) *viticola* nuclear fusion is generally delayed and occurs much later in the resting phase of the zygote. According to Blackwell (1943), the nuclei remain paired but do not fuse for about a month in *Phytophthora cactorum*. Tsang (1929) noted that in *Peronospora effusa* the male and female nuclei are for a time distinguishable. The male nucleus is smaller and stains red with Flemming's triple stain, while the female nucleus stains blue.

In most species of Pythiaceae and Peronosporaceae the fusion nucleus divides when the zygote germinates after a dormant period. McDonough (1946), however, observed in *Sclerospora macrospora* a condition similar in some ways to that described for certain species of the Albuginaceae. Here the fusion nucleus divides immediately after karyogamy, and repeated divisions occur. The mature oospore contains 33 to 44 nuclei as it passes into a resting phase. As in the Saprolegniales it is generally believed that meiosis occurs in the first two nuclear divisions within the germinating zygote, but again no one has presented sufficiently convincing cytological evidence that such is the case. McDonough (1937) has described what appear to be stages in meiotic prophase in the zygote nucleus of *Sclerospora graminicola*, and reduction of the chromosome number apparently occurs in the first division. Some unusual developments were reported to transpire during this division. The nucleolus, instead of degenerating, was found to divide in dumbbell-like fashion, while the nuclear membrane persisted throughout the division and constricted in the middle at telophase. Certain similarities between this and the type of nuclear division reported in the plasmodium of the Plasmodiophorales are obvious.

Bosc (1946) described a similar division, which he compared to promitosis, during vegetative nuclear divisions in *Rhysotheca viticola*. During the division the chromosomes separate into two groups and the nucleolus reportedly divides, the two parts passing into the daughter nuclei. The two parts remain attached by a fine chromatic filament which may persist for a time between the daughter nuclei. The original nuclear membrane persists during the division. However, in the first few nuclear divisions in the zygote the nuclei were found to behave in a typical manner, with the nucleolus and nuclear membrane degenerating. The nuclei return to the promitosis-like divisions at about the 16-nucleate stage. Bosc therefore concluded that the nuclei are diploid up to this stage, that reduction in chromosome numbers occurs between the eight-nucleate and 16-nucleate stages, and that the nuclei from here on are haploid, as indicated by the promitosis-like divisions. Such conclusions do not appear justified by the evidence at hand.

In the Albuginaceae nuclear developments are similar to those in the other two families up to a certain point. Differences are sometimes apparent in the development of sex organs and zygotes. The main types of nuclear cycles in this group and in other Peronosporales have been reviewed by McDonough (1937). Tsang (1929) has confirmed the earlier reports of Stevens on the main types of cytological behavior. In *A. bliti* and *A. portulacae* the nuclei in the antheridium and oogonium undergo two successive synchronous mitoses, the nuclei in the periplasm not undergoing the second mitosis. Many nuclei persist in the oosphere as well as in the antheridium. At plasmogamy about 100 antheridial nuclei are deposited in the oosphere where they fuse with an equal number of female nuclei. Thus a multinucleate zygote (coenozygote) is produced. The nuclei remain in a resting condition until zygote germination. In *A. tragopogonis* the development of the sex organs is at first similar, but nuclear degeneration occurs in the oosphere following the second mitosis, and only one functional egg nucleus remains. One or more antheridial nuclei enter the ooplasm, and a single nuclear fusion occurs, followed by several successive nuclear divisions. The multinucleate oospore passes into a resting phase. In *A. candida*, after the second mitosis, all the nuclei in the ooplasm, except one, pass into the periplasm and degenerate. The antheridium deposits a single nucleus in the

ooplasm and there is a single nuclear fusion. The diploid nucleus undergoes several successive divisions and the resulting oospore is multinucleate as it goes into a period of dormancy. It is believed that in all cases meiosis occurs in the first two divisions of the diploid nucleus, although this has not been definitely established. Tsang has noted that the nuclei of all species studied by him have essentially the same structure and that nuclear divisions are very much the same in all, with one interesting exception. In *A. bliti*, the nucleolus, instead of degenerating during nuclear division, reportedly divides into two parts, one part passing into each daughter nucleus.

Damle (1943) found that the development of the sex organs and fertilization in *A. platensis* are very much the same as in *A. bliti*. The two fungi are quite similar morphologically. Damle observed, however, that they may be distinguished by a few cytological differences, such as differences in spindle orientation in the first mitosis in the oogonium and the absence of a coenocentrum in *A. platensis*. Thirumalacher (1949) studied the cytology of the sex organs and oospores in *A. evolvuli* and found a condition most like that in *A. candida*. The single nuclear fusion which occurs in the egg is followed directly by several successive divisions. The illustrations indicate that the material was not in good enough condition to permit the claim that actual reduction in chromosome number occurs in the second division.

Although it can not be said that anyone has definitely established that meiosis occurs during the first two divisions of the zygote nucleus, this would seem to be the most logical time for its occurrence. It appears that no recent investigators have supported the theory, suggested by certain earlier workers, that meiosis occurs during the nuclear divisions in the developing sex organs.

A number of investigators have attempted estimates of chromosome numbers in various species of the Peronosporales. The same precautions in interpreting these counts must be observed here as in other phycomycetes, since most investigators have expressed considerable uncertainty concerning the exact numbers. Tentative counts (haploid) are listed for the following species: *Phythium deliense*, 4 or 8 (Saksena, 1936); *Phytophthora himalayensis*, 3 (Mundkur, 1949); *Sclerospora graminicola*, 14 (McDonough, 1937); *Rhysotheca viticola*, 14–16 (Arens, 1929; Bosc, 1946);

Peronospora effusa, 6–8, *Albugo bliti*, *A. candida*, and *A. tragopogonis*, 12 (Tsang, 1929); *A. portulacae*, 12 (Tsang, 1929), 8 (Stevens, 1940); *A. evolvuli*, 8? (Thirumalachar, 1949).

MUCORALES. With the aid of a variety of fixatives and staining procedures, Cutter (1942) has studied 15 species of this group cytologically. He found that certain features of nuclear structure and behavior are common to all the species studied by him. The nucleus is provided with nuclear membrane, fine chromatin strands and a nucleolus-like body which he calls the " central body ". Cutter found it to be Feulgen-positive, but he does mention that its positive reaction to the Feulgen test may result from the collapse of a portion of the chromatin reticulum around it. In view of somewhat similar findings in certain higher fungi, the latter suggestion seems more logical. The illustrations indicate that in some cases nucleolus and chromatin are massed together, while in other cases both are quite distinct. The report that the nucleolus-like body divides during mitosis deserves further investigation. In addition to mitotic divisions, a type of division resembling amitosis was reported in older hyphae as well as in maturing and dormant zygotes. However, this appears to be associated with nuclear degeneration and is not considered a normal method of nuclear multiplication.

It should be borne in mind that the nuclei of the Mucorales, like those of most other phycomycetes, are quite minute. Cutter found that the vegetative nuclei range from about 2 to 4 μ in diameter, and the diploid fusion nuclei are not much larger. In all species in which chromosome counts could be made, the haploid number was believed to be 12.

During the course of his study Cutter found four types of nuclear development which he classified mainly on the basis of the cytology of sexual reproduction. In the *Mucor* type, including both homothallic and heterothallic species, the vegetative phase contains only haploid nuclei, plasmogamy occurs between multinucleate gametangia, multiple nuclear fusions occur in the developing coenozygote, and meiosis takes place before the coenozygote enters the resting condition. The conclusion that reduction division occurs at this time is based mainly on the observation that the resulting nuclei are smaller than the original fusion nuclei. Although this appears to be the logical place for meiosis, it is very doubtful that

nuclear size is a dependable criterion for distinguishing between haploid and diploid nuclei. At germination the coenozygote produces either a single sporangiophore or a germ mycelium. Each sporangiophore produces multinucleate spores, often uninucleate in *Absidia*, which in most heterothallic species are all of one compatibility type. Cutter was unable to explain how this is brought about. The subject will be discussed again in connection with other investigations.

In the *Rhizopus* type of development, only a limited number of nuclei were found to fuse in the young coenozygote, the supernumary nuclei degenerating before onset of the resting period. Meiosis is delayed until germination of the coenozygote. Cutter discovered, like most previous investigators, that the coenozygotes of *R. nigricans* do not germinate readily. Although no germ sporangia were obtained, a few abortive germ tubes occasionally appeared. Meiosis was believed to occur at the first divisions of the diploid nuclei. *Absidia glauca*, another heterothallic species placed in this group, has a similar development, but its coenozygotes give rise to germ sporangia, and the spores in any one germ sporangium are of only one compatibility type.

In the *Phycomyces* type, characterized by *P. nitens* and *P. blakesleeanus*, no nuclear fusion appears to occur until germination of the coenozygote, at which time only a portion of the nuclei fuse. The unfused haploid nuclei do not degenerate but pass into the developing germ tube along with the diploid fusion nuclei. Some but not all of the diploid nuclei reportedly undergo reduction divisions in the germ tube or germ sporangium, while others remain unreduced. The multinucleate sporangiospores of the two heterothallic species studied should therefore contain various combinations of "plus", "minus" and diploid nuclei, some containing one, some two, and some all three types of nuclei. Cutter's study of mycelia from the germinated sporangiospores indicates that this is possible. As would be expected many of these mycelia show an apparent homothallism as a result of heterokaryosis or the presence of diploid nuclei which may undergo meiosis at any time. Blakeslee observed this condition earlier. On the other hand, both Sjöwall (1946) and Burgeff (1929) have presented evidence that the only nuclei that enter into spore formation in the germ sporangium of *P. blakesleeanus* are those resulting from meiotic divisions of a single diploid nucleus.

The *Sporodinia* type of development is characterized by *Sporodinia grandis*, an apparently homothallic species. However, although plasmogamy occurs between multinucleate gametangia, karyogamy reportedly fails to take place. Some nuclear degeneration takes place, but the zygospore remains multinucleate as it passes into a dormant condition. The zygospore, upon germination, gives rise to a branched sporangiophore with a number of sporangia bearing multinucleate spores.

The genetic studies of Köhler (1935) on *Mucor mucedo*, in which other genetic factors were used in addition to the compatibility pair, demonstrated that when two pairs of factors are involved in a crossing of two strains, only one of the four possible genotypes will be found in any one germ sporangium. These results led Köhler to conclude that only one fusion nucleus is involved in the development of the germ sporangium. As a further explanation of why all the nuclei in a single germ sporangium are genetically alike, he suggested that nuclear degeneration may occur after the first or second meiotic division so that only one genotype persists. Sjöwall (1946) has recently confirmed Köhler's theory. He observed that, although a coenozygote with many diploid nuclei is produced in *Rhizopus nigricans*, only one of these undergoes meiosis and only one of the resulting tetrad nuclei survives. This nucleus migrates into the developing sporangiophore and divides repeatedly to form all of the nuclei for the sporangiospores, while the superfluous diploid nuclei remain within the coenozygote. Thus, according to the discoveries of Sjöwall and Köhler, the *Rhizopus* and *Mucor* types of development are essentially the same in their genetic implications.

Although it is generally recognized that the sporangiospores of most Mucorales are predominantly multinucleate, Callen (1940) found that in *Rhizopus nigricans* they may be uninucleate or binucleate at the time they are cut out and become multinucleate by further divisions of the nuclei.

Thus far no one has presented convincing evidence of true sexual dimorphism in a heterothallic species of the Mucorales. Several unconvincing attempts have been made to demonstrate differences between the two compatibility types of a species with regard to sporangial size, mycelial color, rate of growth, etc. However, insofar as the present writer is aware, no one has proven that such characteristics represent a true sexual dimorphism. Johannes

(1950) has recently reported on what he calls a secondary sex character in *Phycomyces blakesleeanus*. After studying three different compatible pairs of mycelia, he concluded that the sporangiospores of the plus strains have consistently higher numbers of nuclei than those of the minus strains. However, the differences in number of nuclei are not striking, and a much more extensive study is needed before the significance of these results can be evaluated.

ENTOMOPHTHORALES. Cytological studies on these fungi have not been extensive. Nowak (1930) studied the life history of *Basidiobolus ranarum*. The mycelium is composed of uninucleate cells, and plasmogamy is accomplished between two adjacent cells. Prior to plasmogamy one of the two cells swells considerably and both cells send up beak-like processes in contact with each other. The nucleus in each cell migrates into the beak and divides, a cross wall cutting off the small uninucleate beak cell and leaving the main cell uninucleate. The chromosomes were reportedly too small and too numerous to count. The two main gametangial cells fuse, and the nucleus from the smaller cell passes into the larger cell where karyogamy occurs. Although karyogamy was not actually observed by Nowak, it has been reported by earlier investigators. Woycicki (1927) believed that the fusion nucleus undergoes two successive meiotic divisions, after which three of the resulting nuclei degenerate, leaving a single, presumably haploid, nucleus. This would resemble to a great extent the condition reported by Sjöwall for *Rhizopus nigricans*, and further resembles the developments described for the alga, *Spirogyra*.

Sawyer (1931) studied nuclear divisions in the conidiophores of *Entomophthora sphaerosperma* and reported that the spindle is intranuclear and that the nuclear membrane persists during mitosis. At least 12 chromosomes were believed to be present. The resting nucleus was described as containing one or several nucleoli, two being the usual number. It is probable that the extra "nucleoli" are heterochromatic bodies on the chromosomes. Although azygospores are produced, no true zygotes are formed, and karyogamy probably does not take place in the life cycle of this species. Rees (1932) has reported that *E. fumosa* exists primarily in the form of four-nucleate hyphal bodies within its insect host. The nuclei appear to be typical in structure, except that they were described

as occasionally containing more than one nucleolus. But again the supernumary " nucleoli " are probably heterochromatic bodies. Each hyphal body multiplies by constricting into two hyphal bodies, during which there is a simultaneous mitosis among all four nuclei. When a " conidiophore " is produced by a hyphal body, the four nuclei migrate into it and divide, four nuclei passing into the " conidium ". The zygospore is produced by fusion of two hyphal bodies. Just before plasmogamy occurs, there is a nuclear division in each hyphal body, then one nucleus from each fusing cell passes to the region of copulation where a bud appears. This bud receives the pair of nuclei and enlarges into the zygospore with thickened wall. Apparently, the remaining seven nuclei in each fusion cell degenerate. It is interesting to note that there is no evidence of nuclear fusion in the zygospore and the two nuclei only seem to move further apart. If it is true that karyogamy fails to follow plasmogamy, the sexual process remains incomplete and the zygospores can not be considered true zygotes. This condition somewhat resembles that in *Sporodinia grandis*.

Schweizer (1947), after culturing *E. muscae* in the laboratory for several years and after having studied it cytologically, reported that he never encountered a mitotic figure. He found, however, that the nuclei are typical in structure and that the mycelium is coenocytic. " Conidia ", " conidiophores ", hyphal bodies and azygospores were all observed to be multinucleate. There was no evidence of plasmogamy or karyogamy in the life cycle.

ASCOMYCETES

This class of fungi has furnished a rich field for varied cytological and genetic research. The relatively large size of the diploid nuclei in the asci of many ascomycetes has facilitated study of their structure and behavior during meiotic division, while the genetic importance of the group is enhanced by the fact that it is frequently possible to recover and analyze all the products of the meiotic divisions in order of their occurrence in the ascus. Both homothallic and heterothallic species are common in the group, as well as a considerable number of species which do not fit well into either of these two classifications. The most characteristic type of life cycle from the standpoint of nuclear cytology is that involving a free-growing monokaryotic thallus, a dikaryophase which is

confined to the fructification, and the asci, which are the zygotes and the only true diploid cells in the life cycle. When the ascus nucleus divides, it divides meiotically. There are some notable exceptions to this type of life cycle, such as are found in certain yeasts, which will be discussed under their respective orders. The reader will find the recent review of the cytology of the ascomycetes by Martens (1946) an excellent source of information on nuclear developments in the group.

ENDOMYCETALES. There is considerable variety in the nuclear developments within this heterogeneous group of hemiascomycetes. No system of ascogenous hyphae is present and the dikaryophase is generally lacking. The ascus typically develops directly from a single cell or from the fusion of two cells. Some species are known to have a diploid vegetative phase, a somewhat comparable situation having been found only in one other order of fungi—the Blastocladiales. The most extensive cytological studies have been concerned primarily with the true yeasts, but there have also been a number of cytological investigations of filamentous species.

Varitchak (1928, 1931) studied the nuclear cytology of *Ascoidea rubescens* and found that the hyphal cells and asci are multinucleate. The latter arise directly from the hyphae, and plasmogamy does not occur. Karyogamy involves a single pair of nuclei, and the supernumerary nuclei degenerate. Presumably the first two nuclear divisions are meiotic. Repeated mitoses give rise to many haploid nuclei around which the uninucleate ascospores are cut out. Spindles with centrosomes were described, and the haploid chromosome number was believed to be 2. Varitchak considered these developments indicative of phylogenetic relationships between phycomycetes and ascomycetes. Certainly the peculiar hat-shaped ascospores would seem to indicate a relationship with the Saccharomycetaceae. Walker (1935) also studied *A. rubescens* but was unable to confirm Varitchak's report of karyogamy and meiosis.

Biggs (1937) investigated *Dipodascus uninucleatus* and found that this species, unlike *D. albidus*, which has multinucleate hyphal cells and gametangia, produces uninucleate hyphal cells and uninucleate gametangia. Plasmogamy occurs between two adjacent cells, and the ascus arises directly from the fused pair. Karyogamy takes place in the young ascus and is soon followed by nuclear division, presumably meiotic. Subsequent nuclear divisions are synchro-

nous. Numerous, small, uninucleate ascospores are cut out in the ascus.

Harrold (1950) observed plasmogamy, karyogamy and stages in meiotic division in *Eremascus albus* but was unable to determine the chromosome number. DeLamater and Schwartz (1952), in a study of the same fungus, observed that the nuclear cytology is very similar to that in the higher ascomycetes. Karyogamy follows a sexual fusion between two equal gametangia. The fusion nucleus passes through the characteristic meiotic prophase stages, and at metaphase 6 pairs of chromosomes are present. One unusual feature of this study is the report that the spindle and centrioles are probably of extranuclear origin. The recent studies of DeLamater (1948, 1949*b*) on *Blastomyces* show that the nuclei are typical in structure and in mode of division in comparison with other fungi. DeLamater's staining techniques are worthy of special consideration with the view of applying them more generally in fungus cytology.

Pericystis apis, a unique heterothallic fungus studied cytologically by Varitchak (1933), reportedly shows a tendency towards sexual dimorphism. According to Varitchak, the so-called male mycelium is more branched than the female mycelium and grows more rapidly than the latter. The multinucleate gametangia are at first similar in appearance, but eventually one in each pair becomes an " antheridium " with a fertilization tube, and the other, larger one becomes the " oogonium ". The contents of the fertilization tube enter into the " oogonium ", and there is a pairing of male and female nuclei, followed by karyogamy. The unfused nuclei degenerate. Each fusion nucleus, as it enlarges, has a beaked appearance, and a dense mass of protoplasm is delimited around it to form the " egg ". In each " egg " there occurs a series of nuclear divisions, the first two of which are considered meiotic, and uninucleate spores are cut out in each protoplasmic mass. Each " egg " is compared to an ascus, and the whole structure is considered a " synascus ". Occasionally only one fusion nucleus persists, and only one spore ball is produced in the female cell. The latter type of development was believed to indicate a relationship to a form like *Dipodascus* which may have been derived from such a fungus as *Pericystis* through reduction in the number of spore balls. The cytological developments indicate the relationship of *Pericystis* to both phyco-

mycetes and ascomycetes. It must be admitted that much of Varitchak's work is fragmentary and that there are many details of the proposed nuclear cycle that need further clarification. It is to be hoped that more extensive studies on the cytology and genetics of the organism will be attempted in the future in order to clarify further its cytology and its taxonomic position. It is interesting to note, in passing, that Claussen had earlier found that the spores in each spore-ball showed a sex ratio of 1:1.

The genera *Spermophthora, Nematospora, Eremothecium* and *Ashbya* are discussed here in one group, since they show certain important similarities. All of them produce mycelia and may or may not have budding cells. According to Guilliermond (1928), *Spermophthora gossypii* has an alternation of haploid and diploid generations. Germinating ascospores produce coenocytic mycelia. Later, multinucleate cells are produced by some of the hyphae, and these develop into gametangia. Mitotic divisions occur, and spindles with numerous small chromosomes and polar centrosomes appear. Many uninucleate gametes are cut out in the gametangia and are released by disintegration of the surrounding wall. Gametes fuse in pairs and karyogamy follows, after which a septate mycelium with uninucleate cells and somewhat limited development is produced. The nuclei in this mycelium are diploid. Some of the hyphae produce spherical asci at their tips. Each ascus nucleus divides three times, the first two divisions presumably being meiotic, and eight uninucleate ascopores are cut out. Guilliermond (1936*a*) later observed that his cultures had lost the ability to reproduce sexually, the gametes no longer conjugating but repeating the gametophytic generation. He believed that *Ashbya* and *Eremothecium* might have originated phylogenetically in a similar manner from such forms as *Spermophthora.*

Guilliermond (1927*b* 1928, 1936*a*) observed that sexual reproduction is lacking in *Ashbya, Eremothecium* and *Nematospora,* thus disagreeing with several other investigators who have reported plasmogamy in several species of *Nematospora.* In these genera he found that the asci, or "sporangia" as they are sometimes called, are uninucleate or multinucleate at their origin. After several successive nuclear divisions, which are distinctly mitotic, uninucleate spores are cut out. These spores, upon germination, repeat the mycelial stage on which the asci or sporangia are pro-

duced. Vegetatively, *Nematospora coryli* produces uninucleate budding cells in addition to mycelial filaments, but *Ashbya gossypii* and *Eremothecium ashbyi* produce only a mycelial growth. In *E. ashbyi* intranuclear spindles with polar centrosomes and about 8 chromosomes were reported. Nuclear divisions in the ascus, or " sporangium ", are synchronous. Manuel (1938) found that both plasmogamy and haploid apogamy occur in *N. coryli.* She reported that plasmogamy is followed by karyogamy and that three successive divisions of the fusion nucleus take place. The minute chromosomes observed on the intranuclear spindles could not be counted. It should be noted that actual stages in nuclear fusion were not described or illustrated.

The true yeasts have been studied more intensively than any other group of the Endomycetales. In fact, there can be little doubt that *Saccharomyces cerevisiae* has been more frequently studied from a cytological standpoint than any other fungus. This is doubtless due, not only to the importance of the species in industry, but also to its unusual type of life history.

Both Guilliermond (1940) and Henrici (1941) have outlined what they consider to be the main types of life cycles in the yeasts (Figures 29–31). Guilliermond recognized three groups from this standpoint: " haplobiontic ", " diplobiontic ", and " haplo-diplobiontic " [1]. In the " haplobiontic " type, such as *Zygosaccharomyces,* the vegetative phase is considered to be predominantly haploid, and the diplophase is generally restricted to the zygote, the latter being produced by conjugation of haploid cells. In the " diplobiontic " type, such as *Saccharomycodes,* the vegetative phase is diploid, the haplophase being confined to the ascospores, with plasmogamy occurring among the ascospores in the ascus. In the " haplo-diplobiontic " type, such as *Saccharomyces,* the vegetative phase tends to be diploid, but there is also generally a restricted vegetative haplophase.

Despite the fact that *Saccharomyces cerevisiae* has been the subject of cytological study for over 50 years, investigators at the present time are not in agreement even on what component of the yeast cell is the nucleus. A few recent investigators have returned

[1] It should be noted that these terms are not used in the sense established by Svedelius (Nova Acta R. Soc. Sci. Upsala 4(4): 1–55. 1915) who coined them, and therefore their adoption here is not recommended.

to the old idea of Wager and Peniston that the nucleus is the large cell vacuole and that the body to one side of it is the nucleolus, or centrosome according to Lindegren. The great majority of investigators have concluded that the large vacuole is a cytoplasmic constituent and that the distinctive chromatic body referred to by Wager and Peniston is actually the nucleus. A few fairly recent investigators have concurred with Wager and Peniston that nuclear division in the budding cell is amitotic, but by far the majority report a true mitosis, as did Janssens and Swellengrebel much earlier, the latter having reported the presence of 4 chromosomes in the nucleus of the budding cell.

Kater (1927) studied the budding cells of *S. cerevisiae* and came to the conclusion reached earlier by Guilliermond and others that Wager and Peniston had incorrectly included the cell vacuole in their concept of the nucleus. The "chromatin strands" of Wager and Peniston were considered as metachromatin strands by Kater. He further found that the metachromatin divides into two parts during nuclear division, one part remaining in the original cell and the other part passing into the bud. During nuclear division a spindle with 8 chromosomes was observed, and 8 chromosomes were believed to pass to each spindle pole, followed by migration of one of the two nuclei into the bud.

Guilliermond (1929), in a study of *Saccharomycodes ludwigii*, reported that in no way does the yeast nucleus differ in structure from that of other fungi. However, he described the nucleus as dividing by amitosis. He had previously described a similar type of division in the vegetative cells of other yeasts, although he found that the divisions in the ascus were mitotic in appearance. However, in later years (1936*b*, 1940) he reported that he had observed figures resembling mitosis in the budding cells of *Saccharomyces paradoxus* and *Hansenula saturnus*.

Badian (1937) agreed with Kater that the nucleus does not include the large vacuole in the cell. He did not observe a nucleolus and reported that the nucleus divides by a primitive type of mitosis during which 2 chromosomes split longitudinally and separate, a delicate thread of chromatin remaining between daughter chromosomes as they move apart. Badian thought that the 2 chromosomes in the vegetative cell nucleus were bivalent chromosomes formed by end to end fusion of the 2 pairs of univalent

chromosomes of two haploid cells which had undergone plasmogamy. He believed that this explained his observation of 2 chromosomes in the prophase nuclei of all stages, including meiosis. These findings were reported for *Schizosaccharomyces pombe*, as well as for *Saccharomyces cerevisiae*. Badian's work received unfavorable criticism from both Renaud (1938*b*) and Guilliermond (1940). Guilliermond's publication contains a good review of the more important papers on yeast cytology up to that time, in addition to an outline of the complete nuclear cycle in *S. cerevisiae*.

Renaud (1938*b*) found in *S. ellipsoideus* a metabolic nucleus similar in structure to that described by Guilliermond. During nuclear division the nuclear membrane and nucleolus disappear, as a spindle with small chromosomes and polar centrosomes develops. Eight chromosomes were believed to pass to each pole, each group apearing as a two-lobed mass on the spindle.

Colchicine was used by Richards (1938) with the object of delaying mitosis at metaphase. Instead, according to this investigator, it stimulated growth. When Richards studied his material cytologically, he found no evidence of a true mitosis during budding and concluded that amitotic division was likely. Beams, Zell and Sulkin (1940) concurred with Richards on the occurrence of amitosis in the budding cells. They found that the nucleus is the small body outside the large vacuole. Kater (1940, 1951) has expressed doubt that amitosis ever occurs as a reproductive process in yeasts or in any other organism.

Sinoto and Yuasa (1941) experienced some difficulty in distinguishing the various parts of the resting nucleus, but their concept of the nuclear structure is essentially like that of Guilliermond. They point out that the position of the nucleus on one side of the vacuole causes the combined structures to resemble a large nucleus and nucleolus. Before making their cytological observations, Sinoto and Yuasa used takadiastase to dissolve out many of the interfering granules in the cytoplasm. They found that a true mitosis occurs, with 4 chromosomes passing to each spindle pole.

Most modern investigators, regardless of their diverse opinions on the mechanics of nuclear division and the number of chromosomes present, have agreed that the nucleus is the relatively small body lying outside the large vacuole in the yeast cell. However, in a series of papers and a book, Lindegren (1945*a*, 1945*b*, 1948,

1949, 1951) and Lindegren and Lindegren (1946) have resurrected the old concept of Wager and Peniston. There are numerous weaknesses in Lindegren's theory, many of which are readily obvious, and space does not permit full discussion of them here. But it should be noted that there are gross inconsistencies in the various papers by this investigator and his co-workers in connection with the number, structure and behavior of the supposed chromosomes, in the structure and behavior of the so-called centrosome, and in the concept of the nucleolus. Furthermore the concept of the nucleus as proposed by this group can not be correlated with what is known about the structure and behavior of nuclei in other fungi, nor, in general, can most of the staining techniques used by this group be depended upon to reveal the true nature of the nuclear components.

In connection with the controversy over which component of the cell is the nucleus, several investigators have studied the yeast cell with the aid of specific nuclear stains in an effort to determine the location of the desoxyribonucleic acid. After using the Feulgen technique, Rochlin (1933) found that only the small body which most investigators have called the nucleus takes the stain. The nucleus was reported to measure 0.8–1.3 μ in diameter. Delaporte and Roukhelman (1938) and Delaporte (1939) made a careful study of the nucleic acids of the yeast cell. Their investigations included a study of chemical extracts of the cell. They concluded that thymonucleic acid (DNA) is located in the conventional nucleus and that zymonucleic acid (RNA) is located in the metachromatin corpuscles of the yeast cell. Brandt (1941) studied yeast cells under various physiological conditions with the aid of ultraviolet light and concluded that the true nucleus and the large vacuole are separate structures. He found that this vacuole under certain conditions contains considerable quantities of volutin. He also observed that smaller cells may lack the vacuole entirely, and that starved cells which are kept submerged in water frequently have several smaller vacuoles.

The cytology of *S. cerevisiae* has recently been investigated by a number of Indian workers. Subramaniam (1945, 1946), Subramaniam and Ranganathan (1945*a*, 1945*b*) and Ranganathan and Subramaniam (1947*a*) studied a strain of this yeast and reported a chromosome number of 2 in the nucleus of the vegetative cell.

These workers have reported spindles with centrosomes in the budding cells. However, it should be noted that the illustrations are generally very diagrammatic in appearance and for the most part are not easily correlated with those of other investigators. In fact, it is difficult to understand how the bodies represented as chromosomes by these writers and by Royan and Subramaniam (1951) can be such, in view of the fact that they are described as spherical or sub-spherical bodies with dense periphery and less dense interior. Nevertheless, it is clear that these investigators have not accepted Lindegren's concept of the nucleus, and they have suggested that the bodies identified by Lindegren as chromosomes are actually mitochondria.

Srinath (1946), with the use of a basic fuchsin-toluidine blue combination, claimed that he found 6 pairs of chromosomes in the nucleus of the vegetative cell, while Naidu and Bakshi (1946*b*) observed that 6 chromosomes pass to each spindle pole during mitosis. However, the " chromosomes " are shown widely scattered throughout the cell during nuclear division, and the drawings are so diagrammatic that the conclusions can not fail to be questioned.

Nagel (1946) studied vegetative cells of *S. cerevisiae* with the aid of numerous cytological techniques, including the Feulgen reaction. She called the large vacuole, which she found to be Feulgen-negative, the " magnicorp ", and the small body generally considered to be the nucleus, which she found to be Feulgen-positive, the " parvicorp ". Typical mitosis was not observed, although particulate units were noted in the " parvicorp ". The introduction of these new terms at this time would appear to be entirely superfluous and evasive, and it is not likely that they will gain general acceptance.

Levan (1947) also considered the small body outside the large vacuole as the true nucleus. At mitosis he believed that a diploid number of 10 chromosomes could be counted, a larger number than has been reported by most investigators. However, some of the bodies which he identified as chromosomes are questionable as such, since at metaphase they were described as being as small as 0.1 μ in diameter.

Recently DeLamater (1949*a*, 1950), using a modified basic fuchsin technique, studied budding in *S. cerevisiae*. He claimed that

the Feulgen-positive body lying outside the large vacuole is the nucleus and that it is not connected with the vacuole. Lindegren's work received criticism from DeLamater on the basis that the methylene blue and toluidine blue used by Lindegren are not specific for chromatin. DeLamater studied the nucleus during mitosis and found that 2 pairs of chromosomes, two long and two short ones, appear at prophase, and that the nucleolus degenerates. A small centriole was found to appear and divide, the two parts passing to opposite poles of the developing spindle. The chromosomes divide and a group of 4 passes to each pole. The diploid chromosome number was therefore believed to be 4.

Wingë (1951), whose pioneering genetic studies on the yeasts are well known, has recently presented a review of some of the main concepts of yeast cytology, but the paper is mainly a critique of the work of Subramaniam and co-workers. It is Wingë's contention that most of the latter work is of a superficial nature and is not substantiated by sufficient cytological or genetic evidence. He has presented photographs together with a brief discussion of his own cytological studies, in which he investigated both haploid and diploid cells stained with iron alum haematoxylin and with the Feulgen technique. His figures indicate most often a basic number of 2 stained bodies in the conventional nucleus of both haploid and diploid cells, but occasionally there appear to be 4 in diploid cells. In one of the figures 2 chromosome-like bodies are shown passing into the bud, while 2 remain in the mother cell. As Wingë observed, this description agrees partially with results obtained earlier by Badian. Wingë (1952) has also reviewed the present status of yeast genetics.

While many investigators have studied the cytology of the budding cells of *S. cerevisiae*, few have studied nuclear divisions in the ascus. This is undoubtedly due in part to the difficulty in distinguishing young asci from vegetative cells. The work of Badian (1937), in which it was concluded that a haploid set of 2 chromosomes is received by each of the 4 ascospores, has already been discussed. Guilliermond (1936*b*, 1940) has observed that karyogamy does not always immediately follow plasmogamy in *S. cerevisiae* and in *S. paradoxus*, but that the dikaryotic fusion cell may, by means of conjugate nuclear division, produce dikaryotic buds in which nuclear fusion occurs. Guilliermond has compared this with

the dikaryophase of euascomycetes and basidiomycetes. Renaud (1937, 1938*a*) confirmed Guilliermond's results and, in addition, pointed out that some dikaryotic fusion cells produce not only dikaryotic buds, but also uninucleate buds which may later copulate and produce diploid cells.

The papers of Wingë, the Lindegrens and others on hybridization and segregation in the yeasts have contributed more to an understanding of the meiotic process than have the cytological investigations. These studies have been conducted primarily upon *Saccharomyces* and closely related yeasts, such as *Saccharomycodes ludwigii* and *Zygosaccharomyces priorianus*. Although most of the species investigated are heterothallic, a few are homothallic. Wingë and Laustsen (1939) were not only able to obtain hybrids within a single species, but they were also able to obtain interspecific hybrids in *Saccharomyces*. One of these involved a cross between a homothallic species (*S. chevalieri*) and a heterothallic species (*S. cerevisiae*). In one case hybrids were obtained between haploid isolates of *S. cerevisiae* and *Zygosaccharomyces priorianus*. However, both Wingë and Lietz (1951) doubt that the genus *Zygosaccharomyces* should be held distinct from *Saccharomyces*. Lietz found a typical nucleus in *Z. priorianus*. During budding of the haploid cells, an intranuclear spindle with 3 chromosomes appears, while in the diploid cells, 6 chromosomes are present. In the prophase nucleus of the ascus, 3 bivalents were observed, and the two successive divisions of the nucleus were presumed to be meiotic. It was found that plasmogamy may occur between two ascospores or between two haploid buds. However, Lietz obtained no evidence for the existence of different compatibility types in this species. Plasmogamy and ascus formation were found to occur freely in single-ascospore cultures. Subramaniam (1950) has expressed the belief that *Zygosaccharomyces*, as well as *Schizosaccharomyces*, exists primarily in the diplophase.

A number of recent investigators have claimed that they were able to induce polyploidy in *S. cerevisiae*. Most of these studies seem to stem from the work of Bauch (1942) who reported "gigas" and "super-gigas" races resulting from the use of such chemicals as acenaphthene, camphor and heterauxin. Subramaniam and his co-workers have published numerous papers on this subject. They have suggested that reports by other investigators

of more than 2 chromosomes are due to their having studied polyploid races. Subramaniam and Ranganathan (1946), Subramaniam (1947*a*, 1947*b*, 1948*a*, 1948*b*, 1951), Ranganathan and Subramaniam (1948), Royan and Subramaniam (1952) and others have reported the production of polyploids, some of which resulted from the use of acenaphthene, with chromosome numbers of 3, 4, 5, 6, 8 and 18. Subramaniam (1948*b*), in a paper on endopolyploidy, reported that at the height of fermentation the nucleus almost fills the entire cell, but later decreases in size. Some of the so-called highly polyploid cells were shown to contain dozens of chromosomes. Rao and Subramaniam (1952) have further claimed that these polyploid cells often bud out cells with only 2 chromosomes, instead of the expected polyploid number. However, the illustrations in these various papers are of such a diagrammatic nature and contain so many irregularities that it is difficult to interpret them. Also, as Wingë (1951) has pointed out, the statements concerning polyploidy are not supported by adequate genetic investigations. Lindegren and Lindegren (1951) have recently reported obtaining tetraploid (and triploid) vegetative cells, with segregation during ascus formation giving the expected gametic ratios.

In a paper primarily concerned with a genetic study of mutants of *Nadsonia richteri*, Skovsted (1943) presented a few cytological observations which tend to confirm the earlier cytological work of Nadson and Konokotine on *N. fulvescens* and *N. elongata*. He concluded that karyogamy immediately precedes meiosis and that, following the second meiotic division, three nuclei usually degenerate, leaving one haploid nucleus for the single ascospore.

Naidu and Bakshi (1946*a*) used the toluidine blue technique in a study of the asporogenous yeast *Candida* (*Torula*) *utilis* and reported that the nuclei divide mitotically, with about 4 short rod-like chromosomes passing to each pole. Since no definite nuclear membrane was indicated in the drawings and since the structures identified as chromosomes appear rather widely scattered through the cytoplasm, the results are open to question.

TAPHRINALES. The taxonomic position of this relatively small group of parasitic ascomycetes is in doubt. A number of investigators believe that it is phylogenetically related to the Endomycetales. Dangeard in 1894 was the first to study these fungi cytologically. He demonstrated the occurrence of karyogamy. Various cytological studies have shown that the parasitic mycelium,

which is the chief phase in the genus *Taphrina*, is composed of binucleate cells. Both homothallic and heterothallic species are reported. According to Wieben (1927), *T. epiphylla* and *T. klebahni* are heterothallic and two compatibility types are present. Conjugation between compatible conidia from single ascospore cultures gives rise to the dikaryophase. However, in *T. deformans* (Martin, 1927, 1940; Fitzpatrick, 1934; Mix, 1940) and in *T. johansonii* (Martin, 1927) a homothallic type of development has been reported, in which the dikaryophase arises by the pairing of sister nuclei resulting from the first mitosis in the germinating ascospore or conidium. Single-spore infections are able to produce the complete life cycle. Mix (1935) has reported that occasional conidial copulations were observed in single-spore cultures of *T. deformans*, but he was unable to find any evidence of copulations between conidia from genotypically different monospore cultures.

Eftimiu (1927) studied the cytology of eight species of *Taphrina*, including *T. deformans*, and reported a haploid number of 2 chromosomes in all of them. Some of the illustrations indicate the presence of a larger number. Probably the most detailed cytological study of any species in the group is that of Martin (1940) on *T. deformans*. Martin observed that karyogamy in the subcuticular ascogenous cells is followed by a vegetative nuclear division, during which 8 chromosomes, the diploid number, pass to each spindle pole. Following this division, a uninucleate basal cell, whose contents later degenerate, and a uninucleate ascus are produced. The ascus nucleus then enters meiotic prophase, as evidenced by the appearance of 4 bivalent chromosomes, and the usual three successive divisions occur in the ascus. Eight uninucleate ascospores are produced. The haploid chromosome number is 4. In all these nuclear divisions polar centrosomes, originating from an extranuclear centrosome, were reported for each spindle, but astral rays were not observed. Of course, one of the most unusual features of the cytological developments described by Martin, and one which deserves further study, is the mitotic division of the diploid fusion nucleus. As far as the writer is aware, the only other ascomycetes in which a diploid nucleus has been proved to divide in this manner are certain members of the Endomycetales.

PLECTASCALES. This is generally considered the most primitive group of euascomycetes. The latter are distinguished from the hemiascomycetes in the production of definite fruiting bodies and

in the formation of ascogenous hyphae, in which the nuclei are typically paired, following plasmogamy. In most cases the ascogenous hyphae give rise to the asci by means of the characterisic crozier development which is accompanied by conjugate nuclear division. It seems likely that the crozier process is essentially a method for ascus proliferation. DeLamater (1937) described crozier formation in what appears to be a variety of *Arachniotus aureus* of the Gymnoascaceae. This is probably the most primitive member of the ascomycetes in which croziers have been discovered. The cleistothecium of this homothallic fungus is initiated by a uninucleate antheridium and ascogonium on the same hypha or on different hyphae. The ascogonium winds around the antheridium, while mitotic divisions occur in both, and the two organs fuse at their tips. Nuclear pairing occurs in the ascogonium, and ascogenous hyphae are produced by the latter. Asci are produced in typical fashion, the three customary nuclear divisions following karyogamy.

Several investigators have made cytological studies of various species of Aspergillaceae. Young (1931), in a study of *Monascus ruber,* found that the cleistothecium has its origin in two multinucleate sex organs arising from the same hyphal tip. Plasmogamy takes place between an antheridium and a trichogyne produced by the ascogonium. Nuclear pairing occurs in the ascogonium, and the latter gives rise to the ascogenous hyphae. The asci develop directly from binucleate cells of the ascogenous hyphae without intervention of croziers. Karyogamy in the ascus is followed by the usual three nuclear divisions. The appearance of a phase resembling synapsis in the fusion nucleus was considered evidence that the first division is reductional. Emmons (1932) believed that the asci are uninucleate at origin in *Thielavia sepedonium* and that karyogamy fails to occur.

Wakayama (1931) studied vegetative nuclear division in 13 species of *Aspergillus, A. niger* being the most intensively studied. He described intranuclear spindles with polar centrosomes and with 2 chromosomes as the haploid number. The metabolic or "resting" nucleus was generally found to consist of a karyosome surrounded by a clear area and a nuclear membrane. This type of structure is frequently attributed to vegetative nuclei throughout the fungi. Apparently, as the result of a peculiar sensitivity of

these nuclei to most fixatives, the chromatin collapses around the nucleolus and both stain as one body. A similar condition has been observed in *A. fischeri* (Olive, 1944). The present writer has found that this is less likely to occur with the carmine technique and with certain carefully mixed fixatives in common use. Wakayama's statement that a "nuclear reticulum, if present, appears not to be intimately concerned with the formation of the chromosomes" is considered as evidence that a few of the resting nuclei were properly fixed, but his conclusion can not be accepted. Elisei (1939) reported that in a species of *Penicillium* closely related to *P. lanosum* the conidia, the cells of the conidiophore, and many of the vegetative cells are uninucleate. The haploid number of chromosomes was believed to be 2. The peculiar tendency of a daughter nucleus to degenerate following mitosis is puzzling. Whelden (1940), like Wakayama, considered the normal haploid chromosome number of *A. niger* as 2. However, following irradiation experiments, he reportedly obtained a coarser variant which he later found to have a chromosome number of 4. The cytology of crozier formation in *A. fischeri* was described by Olive (1944). Karyogamy is followed by three divisions in the ascus, but the distinctive details of these divisions were not observed and chromosomes could not be counted. Rees and Jinks (1952), in a study of vegetative nuclear divisions in *Penicillium cyclopium*, estimated the haploid chromosome number to be 6 or 7.

In a cytological study of heterokaryosis in *Penicillium notatum*, Baker (1944) found that the phialides and conidia are uninucleate, while the mycelial cells are uninucleate or multinucleate. When conidia are germinated in groups, the conidia and their germ tubes readily anastomose and the hyphae develop more vigorously. The investigator concluded: "The anastomosing of hyphae and spores in multiple spore transfers assures a combining of factors and heterokaryosis as spores with nuclei of different origins are brought together". Although Baker did not actually demonstrate a single instance of nuclear migration through an anastomosis, her thesis has been supported by later investigations. A later paper by the same investigator (1945) was concerned with conidium formation in *Aspergillus*. Since *A. clavatus*, *A. fumigatus* and *A. repens* all have uninucleate conidia produced by uninucleate phialides, single-conidial transfers may be depended upon to perpetuate

a pure homokaryotic line. On the other hand, *A. echinulatus* has multinucleate phialides and conidia which are multinucleate at their origin. It was therefore concluded that such conidia may be either homokaryotic or heterokaryotic.

Henrard (1934) studied 15 species of *Aspergillus* and found them all to be homothallic. In one species, *A. nidulans*, an interesting phenomenon was recorded. Two types of cultures, designated *A* and *a*, were discovered, and these were found to differ physiologically; that is, when two dissimilar cultures, *A* and *a*, were paired on agar, a red zone formed where the two came together. This did not happen when similar cultures were paired. Furthermore, when perithecia were taken from the line of contact between *A* and *a* cultures, some of them were found to contain a mixture of *A* and *a* ascospores, strongly indicating that hybridization and segregation had occurred in this homothallic fungus. It seems strange that most recent investigators in this field have overlooked this important work.

There have been a number of recent investigations of heterokaryosis in the Aspergillaceae (Gossop et al., 1940; Pontecorvo and Gemmell, 1944; Pontecorvo, 1946, 1949; Pontecorvo et al., 1949; Raper and Fennell, 1948; Roper, 1952; Jinks, 1952*a*, 1952*b*), and at least two of these investigations (Pontecorvo et al., 1949: Roper, 1952) have demonstrated genetically the occurrence of hybridization in ascosporic species, which always appear to be homothallic. Heterokaryons have been obtained with respect to both morphological and biochemical characters. Space does not permit complete discussion of these papers, but a few of the experiments will be mentioned to give the reader an idea of the type of work being carried on with these fungi. Gossop et al. (1940) paired two mutant strains of *A. niger*, a non-ascosporic species, one with cinnamon spore heads, the other with brown spore heads, and obtained heterokaryotic heads that were black like the wild type. Although all the conidia had the same dark color, when plated out they gave rise to the original two mutant type cultures. When the wild type green mold, *A. nidulans*, was paired with a white mutant, heterokaryotic conidial heads with both white and green chains of conidia were produced. Since phialides and conidia are uninucleate, all conidia in a single chain are alike. Pontecorvo (1946) has referred to the first type of gene action as " non-autonomous "

and the second type as " autonomous ". He has also described a similar non-autonomous gene action in *P. notatum*. Pontecorvo et al. (1949) have published a preliminary report on their work with *A. nidulans*, in which they obtained heterokaryons between strains differing in three or more pairs of characters. An analysis of the asci of such heterokaryons revealed the presence of ascospores, not only of the original parental genotypes, but also of various recombinant types. Thus, genetic evidence of a true hybridization in the asci was obtained. These investigators found the haploid number of chromosomes in *A. nidulans* to be 4. Hemmons et al. (1952) studied crosses in *A. nidulans* involving 22 strains, most of which were self-fertile, and found that in a number of cases cross-karyogamy occurred more readily than self-karyogamy, a condition referred to by them as " relative heterothallism ".

Jinks (1952*a*, 1952*b*) has demonstrated the common occurrence of heterokaryosis in wild *Penicillium*. It seems likely that this phenomenon is common in the fungi, particularly in the Fungi Imperfecti, and that it may be of considerable survival value to them.

The history of the development of excellent penicillin-producing strains of *P. chrysogenum* through irradiation experiments from an originally poor penicillin producer, demonstrates the practicality of such experiments in the development of superior strains of useful fungi in the laboratory (Raper and Alexander, 1945).

With the aid of colchicine, Gordon and McKechnie (1945) have claimed that they were able to induce polyploidy in *P. notatum*, with an accompanying increase in yield of penicillin. Sansome and Bannan (1946) were unable to confirm these results. Roper (1952) has presented evidence that camphor may bring about the development of diploid nuclei in the mycelium of *A. nidulans*. He reported that his treatment of a heterokaryotic mycelium with camphor resulted in the production of some diploid heterozygous nuclei which, by means of an ingenious method involving the use of biochemical mutants, could be recovered in some of the conidia. He has presented convincing reasons for considering these cultures diploid, including evidence from a study of recombinant types in the asci and chromosome counts (to be published). Although meiotic divisions readily occur in the asci of diploid cultures, few of the asci mature, but most of those which reach maturity contain

16 ascospores, some eight. Both haploid and polyploid ascospores are produced. The suggestion that the diploid nuclei probably arise in the mycelium by incorporation into one nucleus of two daughter nuclei from two spindles lying side by side is in need of experimental evidence. Pontecorvo (1952) has continued these investigations by studying genetically what he calls " somatic or mitotic crossing over " in diploid heterozygous cultures of *A. nidulans* obtained by Roper's method, and has presented evidence that recombinants may be obtained in the vegetative phase without the intervention of sexual reproduction.

The investigations demonstrating hybridization in these fungi and a few similar studies on other fungi not closely related to these would appear to demonstrate a very important point which is generally overlooked. Whereas homothallism has been looked upon by many as an inferior process which offers little chance for the fungus to vary, these studies indicate that hybridization and segregation in homothallic fungi may prove to be common phenomena. This is probably true not only in the laboratory, but also in nature, and is undoubtedly of great survival value. It is further obvious that, by means of heterokaryosis followed by heterozygosity in the fusion nucleus and by segregation, the homothallic fungus is provided with a means of hybridization very similar to that which occurs in heterothallic fungi. One further advantage is seen in the fact that the fungus is not prevented from completing its life cycle in the homokaryotic condition in the absence of mycelia of other genotypes. This is not to say that all homothallic fungi are capable of hybridizing, as there are at least a few in which this does not seem likely.

A detailed study of the development of the fruiting bodies of certain Elaphomycetaceae was made by Clémencet (1932). *Ascoscleroderma cyanosporum* and several species of *Elaphomyces* were studied, and the developments were essentially the same in all. Plasmogamy was not observed. Asci appear to arise directly from binucleate cells of the ascogenous hyphae without crozier formation. Karyogamy results in the production of a fusion nucleus which reaches a diameter up to 10 μ. Clémencet was somewhat confused about the order of events during meiotic prophase, but he believed that a spireme appears and breaks up into 8 segments which soon become arranged in 4 pairs. The usual three nuclear divisions, the

first two of which are considered meiotic, give rise to eight nuclei, and eight ascospores are cut out, apparently by centrosomes and asters. It should be noted that references to a spireme in the nucleus of the fungi were at one time common, but the term is rarely used today. It is doubtful that concise evidence for the existence of a spireme in the fungi has ever been presented, and it seems that until such evidence is forthcoming, it is best to consider the chromosomes as separate structures during the prophase stages. In any case the interpretation offered by Clémencet can not be considered typical, and part of the text as well as certain illustrations suggest that there are 8 bivalents instead of 4 in the fusion nucleus of this species.

ERYSIPHALES. Most of the cytological studies in this group have been concerned with the Erysiphaceae. Those interested in fungus cytology will already be acquainted with the earlier papers of Harper on the cytology of several species of powdery mildews. It was in these papers that Harper advanced the theory of two nuclear fusions—one following plasmogamy and the other in the ascus—in the life cycle of the ascomycetes. Harper also believed that there are two reductional divisions in the ascus. In spite of the fact that Harper's theory is not generally accepted today, his cytological studies on the group, particularly with respect to the cytology of ascus development, are considered among the most outstanding in the fungi. Later, Gwynne-Vaughan and others elaborated upon the theory of double nuclear fusion and double reductional divisions, chiefly in connection with the study of certain discomycetes. The concepts of Harper and of Gwynne-Vaughan have been discussed and compared in greater detail elsewhere (Aldinger, 1936; Martens, 1946; Olive, 1950), and certain of Gwynne-Vaughan's more recent publications will be reviewed in this paper under the Pezizales.

Most investigators have reported that the nucleus of the powdery mildews is conventional in structure. The comparatively large size of the ascus nucleus has undoubtedly encouraged a number of workers to investigate these fungi cytologically. Eftimiu and Kharbush (1928), Kharbush and Eftimiu (1928), and Eftimiu (1929*a*) have devoted considerable attention to the group, and their studies include species of all the better known genera. Several of these were species previously studied by Harper. These

workers found that the cleistothecium is initiated by two uninucleate branches, the antheridium and the ascogonium. According to them, the antheridium is not functional and plasmogamy does not occur. Harper had earlier reported plasmogamy. The ascogonial nucleus divides several times mitotically, and the ascogonium becomes divided into a row of cells, one of which is binucleate, the others uninucleate. In *Sphaerotheca* and *Podosphaera* the binucleate cell enlarges to form the single ascus directly, whereas in the other genera it gives rise to ascogenous hyphae which produce several asci. Karyogamy was observed only in the ascus. A typical meiotic prophase is followed by the appearance of a distinct spindle with 4 bivalents (Harper reported 8), and conspicuous centrosomes are located at the spindle poles. However, the report that the chromosomes divide transversely in the second and third divisions leads one to question the accuracy of the observations. Uninucleate ascospores are delimited by an astral ray mechanism, as previously described by Harper.

In a study of *Sphaerotheca castagnei,* Moreau and Moreau (1930) observed that the perithecium is initiated in the same manner as described by the foregoing investigators. A convincing comparison was made between the three-celled stage of the ascogonium and a crozier. Karyogamy occurs only in the ascus.

Raymond (1933*a*, 1934) investigated several species of powdery mildews belonging to four common genera, and found apogamous development of the cleistothecia (haploid apogamy) as described by the foregoing authors. In *Microsphaera quercina* he described the bivalent chromosomes as they appear during the various stages of meiotic prophase. The first nuclear division in the ascus was found to be typically meiotic. In all three divisions, 4 (possibly 5) chromosomes reportedly pass to opposite poles. A haploid number of 4 was also believed to be present in the other species studied. Raymond, like several other workers, found that the hyphal cells, haustoria and conidia are uninucleate. He has further claimed that he observed 4 chromosomes during mitosis in the hyphae.

Homma (1934) has reported that the antheridium is functional in *Sphaerotheca fuliginea* and that an antheridial nucleus migrates into the ascogonium where it pairs and fuses with the ascogonial nucleus. Following this, the presumably diploid nucleus divides mitotically several times and the ascogonium becomes divided into

several cells, the uninucleate penultimate one becoming the ascus. Eight chromosomes were believed to pass to each spindle pole during the first division in the ascus. However, it should be noted that neither this nor other important stages in the cytology of the fungus were adequately illustrated. A related species, *S. castagnei*, was studied by Bergman (1941) who disagreed with the reports of Eftimiu and Karbush and the Moreaus that apogamy occurs in this species. He found, as did Homma, that the antheridial nucleus is smaller than the ascogonial nucleus and that the two can be distinguished after they have paired in the oogonium. Descriptions of the mitotic divisions which occur in the ascogonium further emphasize the similarity of the entire ascogonium to a single crozier during this development. The binucleate penultimate cell develops directly into an ascus in which occurs the only karyogamy observed in the life cycle.

In *Phyllactinia corylea*, according to Colson (1938), the antheridium degenerates and the ascogonium develops apogamously. Following mitotic divisions, the ascogonium becomes three-celled in the manner described previously for *Sphaerotheca*, and the middle binucleate cell produces the ascogenous hyphae. The latter do not produce croziers. Karyogamy occurs in the ascus and is followed by the usual three divisions, the first of which is reductional. Colson observed that 10 chromosomes pass to each spindle pole in all three divisions. This appears to be the only paper in which an attempt has been made to distinguish the chromosomes of a powdery mildew morphologically. Accordingly two long pairs, six medium-sized pairs and two short pairs were described by Colson in late meiotic prophase.

There have been few observations on homothallism and heterothallism in the powdery mildews. Yarwood (1931) claimed that *Erysiphe cichoracearum* is a heterothallic species, while Homma (1934) found *Sphaerotheca fuliginea* to be homothallic.

Cytological studies of members of the order other than the Erysiphaceae are comparatively few. Fraser (1935) investigated several sooty molds (tentatively placed in the Erysiphales by Bessey) and concluded that the ascogonium ("archicarp") of *Aithaloderma* and *Capnodium* is a sort of compound structure developed from the fusion of several cells. The nuclei were found to be associated in pairs in the ascogonium, and they were believed to fuse

in pairs there. This suggestion was based, not on actual observation of nuclear fusions, but on the basis of comparative sizes of the nuclei. The method of ascus formation as described here is unique. Ascogenous hyphae reportedly give rise to the asci without crozier formation, and, in view of the fact that no binucleate stage was observed in the young ascus, it was concluded that only one nucleus enters the ascus at its origin. The usual three nuclear divisions occur and eight ascospores are formed. The investigator is at times not too certain of her cytological observations, and it would appear that these species could bear further investigation.

Perisporium funiculatum, according to Beatus (1938), is a homothallic species. Plasmogamy was reported to occur between uninucleate antheridium and ascogonium ("oogonium"). Asci arise directly and without crozier formation from binucleate cells of the ascogenous hyphae. The only karyogamy observed occurs in the ascus. In all three divisions in the ascus, and in mitotic divisions within the ascospores and vegetative hyphae, a haploid chromosome number of 4 was observed.

MYRIANGIALES. The only recent paper on the cytology of these fungi appears to be that of Miller (1938) on a developmental study of *Myriangium duriaei* and *M. curtisii*. An ascogonium, composed of several multinucleate cells in which the nuclei often seem to be paired, a uninucleate stalk and a uninucleate trichogyne, was found imbedded in the young stroma. No antheridium was seen. A primary ascogonial cell divides and gives rise to several multinucleate cells called secondary ascogonia, each of which produces an ascogenous hypha consisting of a chain of binucleate cells. Secondary branches arise from the ascogenous hyphae, and certain binucleate cells of these hyphae eventually give rise to the asci. Conjugate nuclear divisions occur during these developments. Although typical croziers are not formed, a process somewhat resembling crozier formation was reported. In each cell of the ascogenous hypha destined to produce an ascus, a conjugate nuclear division occurs, after which a uninucleate cell is formed at each end and a binucleate cell in the middle. The two nuclei in the middle cell fuse and the cell enlarges to form the ascus. The fusion nucleus enlarges and enters a resting period lasting one to three months, after which it undergoes three successive divisions, and eight ascospores are cut out. Four bivalent chromosomes

were observed during meiotic prophase, and in each of the three divisions 4 chromosomes pass to each spindle pole. The eight ascospores become multicellular, and the asci occur scattered singly throughout the stroma.

HYPOCREALES. Several investigations of considerable interest have been performed on a number of species in this order of true pyrenomycetes, a group which contains a large number of important plant pathogens. Reports of nuclear developments in *Polystigma rubrum* have been conflicting. The Moreaus (1930) have contested the earlier report of Nienberg that plasmogamy occurs between two adjacent cells in the ascogonium. Instead they have stated that there is no plasmogamy and that one or more multinucleate cells of the coiled ascogonium give rise to ascogenous hyphae with binucleate cells, these in turn producing asci by crozier formation.

The genetic studies of Hansen and Snyder (1943, 1946) and the cytological investigations of Hirsch (1947, 1949) have been concerned with various " sexual " strains of *Hypomyces solani* f. *cucurbitae*. Hermaphroditic thalli, which produce conidia and perithecial primordia, are self-sterile but inter-group-fertile, a single pair of compatibility factors being involved. The so-called " male " and " female " cultures were obtained as common mutants from hermaphroditic cultures. The " females " resemble the " hermaphrodites ", but a conidial suspension from such cultures, when transferred to an hermaphroditic culture, will not bring about the production of perithecia. The " males ", on the other hand, produce conidia but lack perithecial primordia. A conidial transfer from " male " to " female " or from " male " to compatible " hermaphrodite " will result in perithecial production, but such conidial transfers to the " male " in reciprocal crosses do not bring about the development of perithecia. In addition to these types, certain cultures called neuters were obtained, but these failed to react sexually in any cross.

Hirsch claimed, in her cytological studies based on the use of material stained by the carmine method and other more recent staining procedures, that the strains are distinguishable on the basis of differences in chromosome numbers. The evidence is somewhat indirect in several instances, since only nuclei in the asci were studied. The haploid chromosome number in typical *Hypo-*

myces solani was believed to be 6. In *H. solani* f. *cucurbitae* it was found that there are 4 bivalents in the fusion nucleus of an ascus produced by a cross between two compatible hermaphroditic strains. Therefore hermaphroditic cultures were concluded to have a haploid number of 4. There are a long chromosome, which is the nucleolus chromosome, two intermediate chromosomes, and a short one. In a cross between " hermaphrodite " and " male " or " hermaphrodite " and " female ", 7 chromosomes were observed at anaphase of the first division, 4 migrating to one pole and 3 to the other. It was therefore concluded that 3 is the chromosome complement of the " male " and of the " female ". It was believed that " male " and " female " each contained a long, a short and an intermediate chromosome. In a cross between " male " and " female ", 3 pairs of chromosomes were said to appear in the fusion nucleus. Although 3 chromosomes were usually found travelling to each pole at anaphase of the first division, in some cases 4 chromosomes reportedly go to one pole and 2 to the other. Thus, the neuters were believed to arise in this manner, and each neuter was supposed to receive a long and a short chromosome.

It should be noted that in no case were chromosome numbers observed in dividing nuclei in the mycelia of these different cultures. Such evidence, if obtainable in these small nuclei, would do much to clarify this problem. It should also be realized that chromosome counts during anaphase in the ascus can be quite hazardous, since the chromosomes of many fungi are notable for their tendency not to separate simultaneously on the spindle. For example, if three pairs had separated and the fourth pair was delayed, this would give the appearance of 7 chromosomes at anaphase. Finally, it is most difficult of all to comprehend how a mycelium can lack two of a normal complement of 4 chromosomes and develop about as well morphologically and physiologically as the mycelium with 4 chromosomes. It is to be hoped that additional cytological and genetic evidence will be brought to bear upon this problem. It appears likely that the situation in *Hypomyces* may prove to have much in common, both cytologically and genetically, with that in *Glomerella.*

With the aid of Barrett's haematoxylin and carmine and orcein staining techniques, Hirsch et al. (1949) have extended their studies to other members of the Hypocreaceae. They have

mentioned the possibility of using such observations from a cytotaxonomic approach. The various species, with their haploid chromosome numbers, are listed as follows: *Giberella lateritium* and *G. roseum*, 6; *Calonectria rigidiuscula* and *Nectria episphaeria* f. *coccophila*, 7.

The cytology of perithecial development in *Nectria flava* was recently studied by Gilles (1947) who found that the ascogonium gives rise to primary ascogenous haphae, periphyses and paraphyses. The primary ascogenous hyphae then produce secondary ascogenous hyphae from which the asci arise. It is not certain whether croziers may assist in ascus production. The haploid chromosome number was estimated at 4.

In *Cordyceps militaris* (Varitchak, 1931) and in *C. agariciformis* (Jenkins, 1934) antheridia have not been observed, and the ascogenous system reportedly arises from the ascogonium without a preceding plasmogamy. In both species a haploid chromosome number of 2 was reported. The eight ascospores, which are uninucleate when delimited, become multinucleate and multiseptate by means of repeated mitotic divisions.

SPHAERIALES. A considerable amount of cytological and genetic work has been performed on various members of this large order of pyrenomycetes. In general, more attention has been given to nuclear developments in the ascus than to cytological details of perithecial initiation, and there are few convincing accounts of the latter process in the literature. Since the description of the genus *Neurospora* by Shear and Dodge (1927), an important literature on the cytology and genetics of the genus has accumulated. The earlier work of Dodge established *Neurospora* as a most valuable subject for such studies. This investigator (1927, 1928) demonstrated the basic cytological and genetic differences between eight-spored and four-spored species of the genus (Figures 48 and 49), pointing out that the eight-spored species produce homokaryotic ascospores and develop in a typically heterothallic manner, whereas the four-spored species produce heterokaryotic ascospores, each of which gives rise to a mycelium on which fertile perithecia are produced.

The claims of Moreau and Moruzi (1931) that a strain of the eight-spored *N. sitophila* could, solely by means of hormonal interplay and without actual contact, induce the formation of fertile

perithecia in a strain of the opposite mating type, and the report of Moreau and Moreau (1930) that asci arise from croziers which in turn are produced by uninucleate ascogenous cells have been discredited by the work of Dodge (1932*a*) and many others. The Moreaus did not believe that plasmogamy preceded perithecial formation in any of the three species which they studied. Dodge (1932*a*) showed that single strains of an eight-spored *Neurospora* are able to produce perithecial primordia (with ascogonial coils) as well as micro- and macroconidia, but that no fertile perithecia develop unless compatible strains are mated. The development of fertile perithecia may be brought about by allowing two compatible strains to come into contact on a Petri dish, or by transferring microconidia or macroconidia from one strain to the other. Backus (1939), in a study of *N. sitophila*, demonstrated the probable manner in which the conidia function in such inter-strain transfers. He observed that macroconidia which were placed on a strain of the opposite mating type frequently fused with conspicuous branching trichogynes which he found arising from the ascogonia. Although no observations were made on nuclear cytology, Backus has convincingly argued that this represents plasmogamy and that nuclear migration from the conidium to the ascogonium initiates the dikaryon.

Schönefeldt (1935) did not observe trichogynes in *N. sitophila* and *N. tetrasperma*. Her claim that anastomoses and possibly nuclear migrations occur between hyphae of opposite strains of *N. sitophila* is not supported by the work of most other investigators. She reported that the ascogonium arises as a coiled multinucleate branch of a vegetative hypha and that it becomes divided into several cells in which the nuclei seem to be paired. It was concluded that nuclear fusion occurs in the ascogonium, since the asci were believed to be uninucleate at their origin. Careful studies by later investigators have shown that such is not the case. Colson (1934) found that the ascogonium of *N. tetrasperma* is composed of several multinucleate cells, but little was learned concerning its ontogeny. The asci were described as arising by typical crozier formation, and nuclear fusion was observed in the ascus.

In view of the considerable amount of work that has been done on *Neurospora*, it is rather surprising that so little is known about cytological developments of the early stages in perithecial formation. In connection with the origin of the dikaryon, a genetic investiga-

tion by Lindegren (1934) demonstrated that the dikaryon is initiated before the ascogenous hyphae are formed and that occasionally more than one pair of nuclei may be involved in a single perithecium. Sansome (1947) confirmed these findings and emphasized the fact that more than one pair of nuclei may be involved in ascus formation within the same perithecium. The failure of Gilles (1950) to find evidence of nuclear pairing either in the ascogonium or in the primary ascogenous hyphae of *N. tetrasperma* may have resulted in part from his exclusive use of sectioned material.

A considerable number of studies have been made on nuclear developments in the ascus. Dodge (1927) studied the three successive divisions in the ascus of *N. tetrasperma* (Figure 49) and demonstrated how, in the third division while the spindles are in parallel pairs, a coordinating centrosome-astral ray mechanism cuts out four dikaryotic ascospores. Occasionally uninucleate spores are cut out and these prove to be homokaryotic in development. Wilcox (1928) found that the spindles of the first two divisions are longitudinally oriented in the narrow ascus of *N. sitophila* and that the third-division spindles tend to be transverse. Eight uninucleate ascospores are cut out by means of a centrosome-astral ray mechanism as described by Dodge.

Colson (1934) found that the fusion nucleus in *N. tetrasperma* is typical in structure. At diakinesis of the first meiotic division, 6 pairs of chromosomes, two long and four very short ones, were reported. In all of the divisions in the ascus and in the mitotic divisions in the ascospores, 6 chromosomes were found to pass to opposite spindle poles. The mode of ascospore formation as described by Dodge was confirmed. Lindegren and Rumann (1938) claimed that *N. crassa* has 9 pairs of chromosomes in its fusion nucleus—two large, two medium, two small, and three very small pairs. When only 6 chromosomes could be counted going to each pole at anaphase, these investigators concluded that the smaller chromosomes had shrunken to invisibility. An attempt was made to count the chromomeres on the various chromosomes during meiotic prophase. Although a total of 155 was counted, it was believed that the number is considerably higher.

McClintock (1945) was the first to study the nuclei of *Neurospora* with the aceto-orcein technique, and her success with this method has stimulated increasing use of this and related techniques

by recent investigators of fungus cytology. McClintock's observations on the chromosomes of *N. crassa* represent one of the most detailed descriptions of fungus chromosomes that the present reviewer has encountered. Following nuclear fusion in the ascus, the chromosomes contract before undergoing synapsis. Eventually 7 completely synapsed bivalent chromosomes are produced. The chromosomes elongate after synapsis and reach their greatest length at pachytene. The longest chromosome, which reaches a length of 15 μ at pachytene and 2.5 μ at metaphase I, is about 2.7 times as long as the shortest. The next to the longest chromosome was found to possess a nucleolus organizer close to the end of its short arm. At late pachytene, each chromosome has a distinct chromomere pattern of constant nature. The smaller chromosomes have from five to seven chromomeres. During diakinesis and metaphase both terminal and interstitial chiasmata appear. The two meiotic mitoses take place, followed by an equational mitosis in the ascus and finally by another equational mitosis in each of the eight ascospores. Heterochromatin was observed in the second meiotic mitosis as well as in the nuclei of the ascospores and vegetative hyphae. McClintock has emphasized the similarity between the nuclear developments in *Neurospora* and in higher plants.

In his study of the chromosomes of *N. tetrasperma* with the carmine and orcein techniques, Cutter (1946) reported finding 12 chromosomes in the pre-synaptic fusion nucleus and 6 pairs—two long and four short ones—following synapsis. One of the longer chromosomes, with its small satellite, was found to serve as the nucleolus organizer. Six chromosomes were believed to pass to each spindle pole during the nuclear divisions in the ascus, as well as during mitosis in the vegetative hyphae. Recently Fincham (1949) made a comprehensive study of the chromosomes of several species of *Neurospora,* including *N. sitophila, N. tetrasperma* (three cultures from widely divergent localities) and an unidentified eight-spored species from New Zealand (Figures 51–53). He found that a method based on the iron alum-carmine technique of Godward (1948) gave superior material for cytological study. In all cases 7 pairs of chromosomes were seen in the fusion nucleus. One of the longer bivalents in each case appeared to be the nucleolus organizer. Certain morphological differences in the chromosome sets of the various species emphasize the practicability of cytogenetic studies in the genus.

Impressive illustrations of the chromosomes of *Neurospora* are found in the few photographs, included in a paper by Dodge, Singleton and Rolnick (1950), of the late prophase nuclei of *N. sitophila* and *N. tetrasperma* stained by the aceto-orcein technique (Figures 54 and 55). These excellent figures leave no doubt as to the presence of 7 chromosomes as the haploid number in both these species. It is now generally accepted that the basic haploid chromosome number for all species of *Neurospora* which have been studied cytologically is 7. It is probably safe to state there has been no more outstanding contribution to the cytology of the fungi than the recent publication of Singleton (1953) on chromosome morphology and the chromosome cycle in *N. crassa* (Figures 50 and 50a). With the use of orcein and carmine, Singleton was able to follow and to illustrate with excellent photographs the cytological developments from crozier formation to mitosis in the ascospores. The majority of McClintock's earlier findings were confirmed, including the observation that the 14 pre-synaptic univalents in the fusion nucleus become conspicuously contracted and that chromosomal elongation does not occur until synapsis has begun. The bivalent nature of the 7 pachytene chromosomes was clearly demonstrated. Each chromosome was shown to contain a series of small chromomeres, as well as several "larger chromomeres" of consistent size and position which assist in its identification (Figure 50). Conspicuous heterochromatic regions were also located on some of the chromosomes. There is no evidence that the so-called "larger chromomeres" are not also heterochromatic. The function of the centrioles in ascospore delimitation has been further elucidated in this important investigation.

The natural occurrence of *N. tetrasperma* as a miktohaplont has been recognized for many years. Whitehouse (1949*b*) has referred to this condition as "secondary homothallism" in recognition of the fact that it is not true homothallism. Basically such fungi are heterothallic. Dodge's cytological studies, showing how two nuclei with opposite compatibility factors are incorporated into the same ascospore, have already been discussed. It should also be noted that Seaver (1937) obtained evidence indicating that about ten per cent of the ascospores in four-spored asci of *N. tetrasperma* are homokaryotic, but this percentage appears to be much too high in view of the data presented by other investigators. In an analysis of certain data published by Dodge and co-workers,

Sansome (1946*b*), following up a suggestion by these authors, concluded that the locus for heterothallism in this species is close to the centromere. Thus it segregates predominantly in the first meiotic division. This was later confirmed by Dodge et al. (1950), whose investigations show that crossovers between the compatibility locus and centromere are very rare. Dodge (1927, 1935*b*) demonstrated in *N. tetrasperma* the occasional occurrence of spores which are uninucleate at their origin and therefore homokaryotic. Cultures from such spores did not produce perithecia when grown alone, but when compatible cultures were paired, the hyphae anastomosed, nuclear migrations occurred, and the vegetative hyphae continued their growth in the heterokaryotic condition, eventually giving rise to fertile perithecia. Dodge (1942*a*) was further able to demonstrate a phenomenon which he termed " heterokaryotic vigor " in *N. tetrasperma.* When he combined a homokaryotic dwarf mutant, which grew slowly and produced few conidia, with another dwarf mutant, he obtained a heterokaryotic mycelium which grew much more vigorously and produced many more conidia than either of the mutant cultures alone. This condition is distinguished from true hybrid vigor on the basis that diploid nuclei are not present in the mycelium.

Gilles (1950) has recently demonstrated cytologically that the mycelium produced by a normal heterokaryotic ascospore of *N. tetrasperma* is composed of cells, mostly multinucleate, in which there is no evidence of nuclear pairing. Uneven numbers of nuclei were frequently found in the mycelial cells, as well as in the macroconidia, and conjugate nuclear divisions were not observed.

There are numerous papers by Beadle, Tatum, Sansome and others which have conclusively demonstrated that heterokaryons may be obtained in the eight-spored species of *Neurospora* by bringing together biochemically deficient mutants of the same compatibility type. The reader is referred to the review paper by Beadle (1947) for an account of the outstanding work being carried on in this field, which has come to be known as " biochemical genetics ". He will undoubtedly be impressed by the extent to which these fungi have contributed to the general store of knowledge concerning the nature of gene action.

The results obtained by most investigators who have worked with biochemical mutants have led them to conclude that, while

heterokaryosis involving nuclei of opposite compatibility types is normal in *N. tetrasperma*, such heterokaryons do not occur in the eight-spored species (Sansome, 1945, 1946*a*). However, a very recent investigation by Gross (1952) has proved that, with certain biochemical mutants, heterokaryons can be obtained between opposite compatibility types in *N. crassa*. Although such heterokaryons contained very uneven nuclear ratios, they always produced perithecia, and isolation of ascospores from these perithecia gave the expected segregations.

Lindegren (1932, 1933, 1936) and Lindegren and Lindegren (1937, 1939) have been primarily responsible for demonstrating genetically that the first nuclear division in the ascus is reductional; that is, it is the division in which homologous centromeres separate; whereas genic segregation may occur in the first division or, as a result of crossovers, in the second division. Segregation for the pair of genes controlling compatibility in *Neurospora* occurs considerably more frequently in the first division than in the second. There is no valid evidence for a segregation of factors in the third (equational) division. Excellent discussions of crossing-over and second division segregation in the fungi will be found in papers by Dodge (1940) and Ryan (1943). With the aid of several linked factors, the Lindegrens attempted to map the compatibility chromosome of *N. crassa* which they concluded to be the longest one. Recently data have been accumulated by Houlahan, Beadle and Calhoun (1949) to show that 69 genes representing at least 35 loci may be placed in five linkage groups. St. Lawrence (1952), in an excellent cytogenetic study of inversions in *N. crassa*, has connected several linkage groups with specific chromosomes. In contrast to the findings of the Lindegrens, it was concluded that chromosome 6, the next to the shortest, carries the compatibility locus in its short arm.

Dodge, Singleton and Rolnick (1950) recently studied the effects of a dominant lethal gene upon ascus abortion in *N. tetrasperma* and found that on a certain medium the asci, instead of aborting as usual, formed 8 ascopores. Preliminary cytological studies indicate that the normal conjugate pairing of the spindles in the ascus has been interfered with, and that the spores are cut out much as in *N. crassa* or *N. sitophila*. It would be interesting to know more about the cytological details here, particularly with

reference to the astral ray mechanism which cuts out the spores. Here a single gene change has caused the disappearance of the most obvious difference between a four-spored and an eight-spored species. In this and in other papers Dodge (1934, 1935*a*, 1939*b*) and Dodge and Appel (1944) speak of recessive and dominant lethals for ascus abortion and of asci homozygous and heterozygous for such factors. Although the ascus with its fusion nucleus may be considered a true hybrid, it must be borne in mind that the actual abortion of the ascus does not occur until the fusion nucleus has divided several times and only haploid nuclei are present in the ascus. Therefore, the question arises as to whether this is a true hybrid effect or, at least in part, a heterokaryotic effect. In actual practice, however, such a technical distinction is hardly necessary or practical.

Evidence of " maternal inheritance " was recently obtained in *N. crassa* by Mitchell and Mitchell (1952). When a certain slow-growing mutant was used as the maternal parent in a cross with wild-type, only slow-growing progeny resulted; when the mutant was used as the fertilizing parent almost the entire progeny grew normally.

The genus *Gelasinospora* contains both eight-spored and four-spored species and resembles *Neurospora* very closely except that the conidia are lacking. The cytology of ascus development in *G. tetrasperma* resembles that of *N. tetrasperma* in practically every essential feature (Dowding, 1933; Dodge, 1937). It seems fairly obvious that these two genera are phylogenetically closely connected. Dowding and Buller (1940) experimented with heterokaryons in *G. tetrasperma* and made observations upon the rate of nuclear migrations that occur after anastomoses between two homokaryotic strains of opposite compatibility types. They concluded that the migration of nuclei of one type through the mycelium of the other type is independent of cytoplasmic streaming, and that the rate of nuclear migration is two or three times the rate of elongation of the hyphae. The nuclei were found to move through small central pores in the cross walls.

In *Sordaria fimicola*, a homothallic species, Piehl (1929) found that the perithecium arises from two branches that coil about each other, but the two were not reported to fuse. Cell fusions were believed to occur in the coil which gives rise to the ascogenous

hyphae. Asci arise by crozier formation, and nuclear fusion occurs in them. At the end of the third division in the ascus, eight uninucleate spores are cut out. Ritchie (1937) has reported, in the same species, that no antheridium is present and that the perithecium is initiated by the appearance of a coiled " archicarp ". The report that nuclear fusion occurs in the ascogenous hyphae and that the ascus is uninucleate at its origin deserves reinvestigation. The eight ascospores appear to be delimited by a centrosome-astral ray mechanism. Page (1933) has studied the life history of what she considers a four-spored form of *S. fimicola.* The fungus is a miktohaplont and resembles *Gelasinospora tetrasperma* very closely. Dengler (1937) studied three homothallic species of *Sordaria* and found that in all three the asci typically arise by crozier formation. In *S. macrospora* plasmogamy was found to occur between multinucleate ascogonium and antheridium, whereas the antheridium was functionless in *S. brefeldii* and completely lacking in *S. uvicola.* Although ascus development is normal in *S. macrospora,* unique developments were observed in *S. uvicola* and *S. brefeldii.* In *S. uvicola* one of the two nuclei in each young ascus was found to degenerate without fusing in about twelve percent of the asci, while in *S. brefeldii* one nucleus consistently degenerated, and karyogamy did not occur. In all cases eight uninucleate ascospores are formed, and these become binucleate after a mitotic division of each nucleus.

The investigations of *Pleurage anserina* by Dowding (1931), Ames (1934) and Dodge (1936) reveal a life cycle similar in its essential details to that of *Neurospora tetrasperma* and *Gelasinospora tetrasperma.* Since any homokaryotic isolate (obtained by selecting the occasional uninucleate ascospores which appear) produces both ascogonial coils and " spermatia ", but is self-sterile and must be mated with a compatible isolate if perithecia are to be produced, the two types of isolates were found to differ " not in sex but in compatibility " (Ames, 1934). It seems that a similar reasoning would apply to most other heterothallic fungi and that, unless it can be proved that a true sexual dimorphism is involved, it is probably best to refer to the genes controlling interfertility as compatibility (or mating type) factors rather than as sex factors. Undoubtedly the sexual process as a whole is influenced by many different genetic factors.

The special theory of nuclear behavior postulated for *P. anserina* by Rizet and Engelmann (1949) to fit their data on segregation of the compatibility factors—a theory involving a most peculiar crossing-over mechanism heretofore unknown—can not be accepted as valid, particularly in view of the fact that the cytology of this species has never been adequately described.

Rosellinia reticulospora, a homothallic species, has been found capable of producing perithecia either by haploid apogamy or by means of plasmogamy between an antheridium and an ascogonium (Greis and Greis-Dengler, 1940). Fusion between a false trihcogyne and a vegetative hypha was also reported. Ascogenous hyphae give rise to asci through crozier formation. Zickler (1934, 1937) studied the genetics of *Bombardia lunata*, and, although cytological details are lacking, it appears that the life cycle of this heterothallic species is quite similar to that of an eight-spored *Neurospora*. Zickler concluded from his study of segregation of compatibility factors (a single pair) and of certain mutant characters that disjunction of the centromeres must occur part of the time in the second meiotic division. In a re-analysis of Zickler's data, Ryan (1943) has presented a plausible explanation based on the occurrence of first division disjunction of centromeres and crossovers. He states: "Thus there is every reason to believe that, in molds, as in other organisms, homologous kinetochores separate in the first meiotic division (reductionally) and each kinetochore divides in the second division (equationally)". Catcheside (1944) has reinvestigated the part of Zickler's experiments dealing with the inheritance of factors controlling ascospore color. His observations on "biased segregation" in the ascus are of considerable interest.

In *Chaetomium kunzeanum* and *C. botrychoides* (Greis, 1942) plasmogamy occurs between an ascogonium and an antheridium or vegetative hypha. Following plasmogamy in *C. kunzeanum*, the ascogonium may ramify and give rise to several perithecia. Croziers reportedly do not occur in either species. The haploid chromosome number in *C. kunzeanum* is 8, in *C. botrychoides*, 6.

A number of investigations of various species of the genus *Ophiostoma*, formerly included in *Ceratostomella*, have been made in recent years. Varitchak (1931) found that the ascogonium of *O. piceae* develops apogamously and, after becoming multinucleate,

gives rise to ascogenous hyphae from which the asci arise in typical fashion by crozier development. Three nuclear divisions follow karyogamy, and eight uninucleate ascospores are cut out in each ascus. As in all species of *Ophiostoma*, the ascus wall deliquesces very early. Mittman (1932) observed that single-ascospore cultures of *O. fimbriata* produce perithecia. She found no evidence of an antheridium and concluded that one of the inner cells of the ascogonial coil becomes binucleate, then multinucleate, by mitotic divisions of its nucleus. This multinucleate cell then divides into several cells, each with two or more nuclei, which in turn give rise to ascogenous hyphae with binucleate cells. Although crozier formation was not described, some of the illustrations indicated that croziers may be produced. Karyogamy is followed by the usual three nuclear divisions in the ascus. At the end of the second division, the protoplasm in the ascus was described as cleaving into an inner and an outer zone, the outer forming the ascus wall and the inner furnishing the cytoplasm of the ascospores following the third division. Mittman also studied *O. coerulea* and found it to be heterothallic, apparently with a single pair of compatibility factors. Although the origin of the dikaryon was not determined, the description of a branch from a neighboring cell entering into the ascogonial coil indicates a development similar to that described in *Glomerella*.

The description of perithecial and ascus development in *O. fimbriata* and *O. moniliformis* by Andrus and Harter (1933, 1937) is extremely confusing and not interpretable on the basis of what is known to occur in other fungi. The origin of the binucleate condition is left in doubt. According to their second publication, crozier formation occurs. The behavior ascribed to the fusion nucleus is unparalleled elsewhere in the study of the fungi. The three successive divisions reportedly occur in the nuclear vacuole while the original membrane of the fusion nucleus expands to become the thin membrane enclosing the ascospores. This membrane breaks down very early, and no true ascus wall appears. The conclusions that 2 unequal chromosomes represent the diploid number, that reduction does not occur in the ascus but probably at the time of ascospore germination, and that dividing haploid nuclei show a single chromosome going to each pole appear to be based on a study of inadequately differentiated material. Such conclusions

have been refuted by the recent genetic work of Olson (1949). Andrus (1936) also studied *O. multiannulata*, a heterothallic species, but was unable to identify clearly the chromosomes. In their study of *O. fimbriata*, Gwynne-Vaughan and Broadhead (1936) found no evidence of plasmogamy and concluded that the binucleate condition arises in a cell of the ascogonium by a mitotic division of its original haploid nucleus. No nuclear fusions were reported in the ascogonium, which eventually gives rise to ascogenous hyphae. The latter produce asci by typical crozier formation. Three bivalent chromosomes were counted at prophase of the first nuclear division in the ascus. These authors found that the ascus has a true cell wall which is not the product of the expanded nuclear membrane, and they have further suggested that the large "nuclear vacuole" observed by Andrus and Harter is actually only a cell vacuole which is often present and which "may produce the illusion of enclosing the nuclei of the ascus". Olson (1949) has described a situation in *O. fimbriata* which in many respects resembles that described in *Glomerella*. He found that, although self-fertile strains are common, perithecia from these strains frequently give rise to ascospores which germinate to produce both self-fertile and self-sterile strains. Crosses between certain strains, including some self-sterile ones, produced perithecia containing ascospores of both genotypes, thus indicating that plasmogamy precedes perithecial development. Hutchinson (1950) found that in *O. majus* a nuclear vesicle is not involved in the formation of the ascus wall, which deliquesces during ascospore formation.

In *Ceratocystis* (*Ceratostomella*) *piceae*, a homothallic species, and in *C. autographa*, a heterothallic species, Bakshi (1951) found that the asci are produced by typical crozier formation. However, there are some discrepancies in the account of meiosis and it is probable that the haploid chromosome number is 4 instead of the reported 2.

In a detailed study of the morphology, cytology and parasitology of *Gnomonia ulmea*, Pomerleau (1938) has reported that ascogenous hyphae with binucleate cells arise from multinucleate cells of the coiled ascogonium and that the asci are produced by typical crozier formation. Nucleolar fusion was observed during karyogamy. In all three divisions in the ascus centrosomes were observed

at the spindle poles, and 2 chromosomes were reported to pass to each pole. However, some of the figures strongly indicate that the haploid chromosome number may be 4 instead of 2. For example, in one late anaphase figure, where 4 chromosomes were observed at each pole, it was concluded that the original 2 chromosomes had merely split prior to the next division. In view of what is known to occur in other fungi, this interpretation is open to criticism. The eight ascospores are cut out by a centrosome-astral ray mechanism.

Edgerton, in his publications on *Glomerella* in 1912 and 1914, was probably the first to demonstrate hybridization in the higher fungi. The genetics and life history of *G. cingulata*, including a discussion of homothallic and heterothallic tendencies of the various strains, were reviewed recently by Olive (1951). Whitehouse has referred to the situation in *Glomerella* as " partial heterothallism ". The group of investigators at Louisiana State University, including Edgerton, Chilton, Wheeler and others, have been primary contributors in this field. Only the cytological developments will be reiterated here (Figures 32–47). The perithecium has its origin in a pair of uninucleate initials which arise as short branches from adjacent cells of a hypha. Although Hüttig (1935) considered these to be antheridium and ascogonium in *G. lycopersici*, the later work of McGahen and Wheeler (1951) indicates that this is not the case. By making observations on living cultures as well as upon material stained with the carmine method, these two investigators found that one of the two initials surrounds the other, the inner one becoming the ascogonial coil and the outer one forming the perithecial wall. Before the perithecial wall has completely surrounded the ascogonial coil, a copulation hypha, or antheridium, grows into the perithecial primordium from outside and fuses with the tip of the ascogonium, thus initiating the dikaryophase in the ascogonium. In a self-fertile strain grown alone, the copulation hypha typically develops from a nearby cell of the same hypha bearing the initials. In a cross between two compatible strains, the perithecial initials arise from the hypha of one strain, while the copulation hypha is produced by the other strain. An example of the latter is found in the cross betwen the self-fertile clumped-perithecial culture and a conidial culture, which is self-sterile. Plasmogamy has been observed between a copulation hypha produced by the conidial culture and an ascogonium produced by the

clumped perithecial culture. It is interesting to note that some perithecia appear in which all the ascospores are of the conidial type. There is reason to believe that the conidial culture in such cases has been stimulated by the fertile strain to produce perithecia of its own, although it never does so when grown alone.

Following plasmogamy, the ascogonium is found to be composed of a varying number of cells, some of which are binucleate (Wheeler et al., 1948). Apparently, crozier proliferation starts with only one of these cells, and all the asci in a perithecium develop from proliferations of this one cell. In all stages thus far described, the nuclei were found to be typical in structure, containing a distinct nucleolus, chromatin strands and nuclear membrane. Karyogamy occurs early in the young ascus. The two nucleoli come together and fuse during synapsis of the nucleolus chromosomes, which are morphologically distinct from the others. The nucleus enlarges greatly, and its chromosomes become very extended and sinuous as the ascus enlarges. This is probably a metabolic phase which accompanies growth of the ascus. During late prophase and at metaphase of the first meiotic division, there appear 4 bivalent chromosomes—one long (the nucleolus organizer), two intermediate, and one short. During all three divisions in the ascus, 4 chromosomes pass to each spindle pole (Lucas, 1946; Wheeler et al., 1948). Eight haploid uninucleate ascospores are produced. The nucleolus disappears during the first division, and nucleoli do not reappear until the ascospores are cut out. The cytological investigations further confirm the conclusions reached through genetic studies that the differences observed among the various strains are not determined by differences in chromosome numbers, as has been claimed for *Hypomyces*. Genetic studies have demonstrated that the distinguishing features of the various strains are controlled primarily by allelomorphic factors located at two loci on a single chromosome.

Few pyrenomycetes have been studied in greater cytological detail from the beginning of perithecial initiation to ascospore formation than *G. cingulata*. The early stages in perithecial initiation are notably difficult to interpret in most of these fungi, and it is not surprising that so many divergent descriptions of it have been published. There can be little doubt that considerable variation in mode of perithecial formation exists among the various pyrenomy-

cetes, but it also appears to be almost customary to consider any two initials arising side by side on a hypha, as described for *Glomerella*, as ascogonium and antheridium. The antheridium is variously described in the literature as functional or non-functional, and there are often numerous conflicting reports for the same species. A reinvestigation of these species is greatly needed, particularly in such groups as the powdery mildews, the Hypocreales and other genera of the Sphaeriales.

Varitchak (1931) reported that in *Xylaria polymorpha* a single septate and multinucleate ascogonium develops in each perithecial fundament and gives rise to ascogenous hyphae with croziers. The asci develop in typical fashion and eight uninucleate ascospores are cut out in each.

There have been very few cytological reports on pyrenomycetous lichens. Stevens (1941), in his study of *Dermatocarpon aquaticum*, observed that the asci arise in typical manner by crozier formation. Spindles with very distinct centrosomes, each composed of several smaller granules, were reported during the three successive nuclear divisions, following which eight uninucleate ascospores are produced. In all divisions the number of chromosomes passing to each pole was believed to be 8.

PSEUDOSPHAERIALES. The cytology of ascus development in *Venturia inaequalis* has been studied by Backus and Keitt (1940) who found in the ascus a large fusion nucleus with nuclear membrane, nucleolus and sinuous chromatin strands with beaded appearance. The first-division spindle is oriented vertically and has polar centrosomes, while second- and third-division spindles may be oriented in various directions in the ascus. Nevertheless, the evidence indicates that there is little tendency for spindles to ovrlap in any of the divisions, so that the meiotic products are arranged in serial order following the third division. Genetic studies hav' confirmed these cytological observations. During the divisions i the ascus the nucleolus disappears and a new one reappears in each newly formed nucleus. The haploid number of chromosomes was estimated to be from 4 to 6. Neither this cytological study nor the excellent genetic work of Keitt and his coworkers supports the earlier report by Killian that there are two different nuclear fusions (one in the ascogonium and one in the ascus) in the life cycle of *V. inaequalis*.

A most unusual development has been reported in *Mycosphaerella tulipiferae* by Higgins (1936) who found that uninucleate sperm cells, usually four in number, are produced within each sperm-mother-cell in the spermogonium. The archicarp is composed of uninucleate "oogonium" and a trichogyne, and plasmogamy is accomplished by fusion between spermatium and trichogyne. The spermatial nucleus supposedly migrates through the trichogyne to the ascogonium ("oogonium") and pairs with the nucleus in the latter. Conjugate nuclear divisions occur, and ascogenous hyphae arising from the ascogonium produce asci by crozier formation. Thus, plasmogamy in this species resembles in some ways that described later by Backus (1939) in *Neurospora.*

HYSTERIALES. The ascocarp in this group has been termed a "hysterothecium", which is considered by some to be an elongated perithecium, while others regard it as an apothecium. Jones (1935) studied the development of the hysterothecium in *Lophodermium pinastri,* which causes a "leaf cast" of pines, and found that the ascigerous stage is typically preceded by "spermogonia". Some of the "spermogonia" were found to contain "oogonia" with trichogynes, although ascocarps might also arise independently of the "spermogonia". The presence of two nuclei in some of the "oogonia" led Jones to suggest that one of the nuclei was derived from a "spermatium". However, plasmogamy was never actually demonstrated. Until this has been observed here, as well as in other fungi with similar structures, it is probably advisable to call the conidial stage a pycnidium and the conidia, pycnidiospores. Eventually, a chain of multinucleate ascogonial cells appears in the young ascocarp, and from these the ascogenous hyphae with paired nuclei develop. It was stated that asci may be produced with or without the aid of croziers. Several other investigators have reported similar results in a number of ascomycetes when using sectioned material. Such material is frequently misleading. Smear preparations are in general far superior for observing crozier development. Jones observed that karyogamy in the ascus is followed by the usual three nuclear divisions, after which eight slender uninucleate ascospores are cut out.

HEMISPHAERIALES. There have been few recent cytological studies in this group which is generally considered intermediate between pyrenomycetes and discomycetes. In a study of *Stigmatea*

geranii, Holm (1952) observed that there is no dikaryophase in the ascogenous hyphae and that the asci may be formed with or without the aid of croziers. The haploid chromosome number was found to be 4.

HELOTIALES. The life histories of a number of these inoperculate discomycetes have been studied in detail. The cytology of ascus development in *Coccomyces hiemalis* was studied by Backus (1933), who described the asci as arising by means of typical crozier formation. The fusion nucleus, which reaches a diameter of about 7.5 μ, has a conspicuous nucleolus and beaded chromatin strands. Four chromosomes, presumably bivalent at metaphase I, appear on the metaphase spindle in all three divisions in the ascus. The eight ascospores are cut out by astral rays. In a cytological study of *Pseudopeziza medicaginis* and *Arachnopeziza aurelia*, Raymond (1934) found that 4 chromosomes, the haploid number, pass to each spindle pole in all three divisions in the ascus.

The most intensively studied members of the Helotiales are a number of species which were earlier classified in the genus *Sclerotinia* but which have recently been segregated into several smaller genera by Seaver and others. A peculiar method of ascus formation has been described by Kharbush (1927*a*) for *Botryotinia fuckeliana*. The young binucleate ascus is reportedly formed by the fusion of two hyphal branches in the mycelium. At the beginning of karyogamy, the chromatin strands are considerably contracted, lengthening later in the enlarging fusion nucleus, as described more recently by McClintock (1945) in *Neurospora*. It is quite difficult to deduce from Kharbush's statements exactly how many chromosomes are present, but a study of the illustrations indicates that the haploid number is 4. Heuberger (1934) observed crozier formation in *Monilinia fructicola*. He found that a haploid number of 4 chromosomes passes to each pole in all three nuclear divisions in the ascus. A more definitive staining technique would probably have demonstrated that there are no basic differences among the three (four, if the fusion nucleus is included) types of nuclei said to be present in various parts of the fungus.

Croziers were not observed by Nardi (1930) in *Sclerotinia tuberosa*, and the asci are reportedly budded out by binucleate subterminal cells of the ascogenous hyphae. The fusion nucleus produces a spindle with centrosomes, while the nucleolus and nuclear membrane disappear early. A haploid chromosome number of 8

was reported. Frandsen (1946), in a study of *Sclerotinia trifoliorum*, found that ascogenous hyphae with paired nuclei appear early in the stalk of the developing apothecium, but no sex organs were observed. The ascogenous hyphae were found to have clamp connections resembling those of basidiomycetes, as described by Greis (1938) for the Tuberales. Terminal binucleate cells of the ascogenous hyphae develop into asci. At prophase of the first division, densely staining extranuclear bodies of uncertain origin were observed in the cytoplasm outside the fusion nucleus. A spindle with centrosomes and 8 bivalent chromosomes develops, and 8 chromosomes were believed to pass to each pole in this and the following two divisions in the ascus. The same number of chromosomes was also reported during mitosis in the vegetative hyphae. Björling (1951) also studied the cytology of *S. trifoliorum*, and his observations in general confirm those of Frandsen. Considerable attention was given to the extranuclear bodies in the ascus, although their origin was not determined. Björling believed that the haploid chromosome number is 6, although he admitted that the chromosomes were difficult to count. Both Frandsen and Björling (in 1951, as well as in a publication prior to Frandsen's) made some very interesting observations on variation in this species. Both found the fungus to be essentially homothallic. However, distinct biotypes were found which, when paired in the laboratory, gave rise to apothecia, some of which apparently contained hybrid asci. These apothecia were produced by sclerotia formed on the line between paired cultures. Frandsen observed anastomoses between germ tubes of different ascospores, as well as between hyphae of two cultures of different genotypes, and suggested that heterokaryons may arise by such means. These findings closely parallel those of Henrard (1934), Pontecorvo et al. (1949) and Roper (1952) concerning the occurrence of hybridization in homothallic Aspergillaceae. Björling further observed in the asci of *S. trifoliorum* a phenomenon resembling genic segregation but not demonstrable as such by genetic analysis. He found that when apothecial caps were severed from their stalks for varying periods prior to spore maturation, after which they were fixed and stained, many of the asci had four spores staining darkly and four staining lightly. These could be arranged in all kinds of sequences, including alternate arrangement. Since these results were obtained in homo-

karyotic as well as heterokaryotic cultures, they were not believed to be explicable on the basis of genic segregation. It was suggested that the results may have been due to the effect of severance of the apothecium upon the apportionment of nuclear (non-genic) or extranuclear materials among the ascospores.

Eftimiu (1929*b*, 1929*c*, 1933) has studied the cytology of ascus development in *Geoglossum hirsutum, Spathularia flavida* and *Mitrula phalloides.* Nuclear details were found to be essentially the same in all. The intranuclear centrosome divides into two bodies prior to spindle formation. Spindles with polar centrosomes appear in all three divisions in the ascus, and the haploid chromosome number in all three species is probably 4. The ascospores are cut out by means of a centrosome-astral ray mechanism.

PEZIZALES. Numerous species of this large group of operculate discomycetes have been subjected to cytological investigation. A diagrammatic representation of nuclear developments in a typical heterothallic discomycete is presented in Figure 59. These fungi are among the most desirable for cytological study, since the asci and their fusion nuclei are, as a rule, relatively large. Although many of them at first appear to be ideal for genetic work, the frequent failure to obtain good germination of ascospores or difficulties in inducing cultures to fruit in the laboratory have impeded genetic progress. Studies conducted in our laboratory at Columbia University have succeeded in eliminating these obstacles for a number of interesting species. The results will be published shortly.

Harper's theory of two nuclear fusions in the life cycle of ascomycetes has already been discussed in connection with the powdery mildews. Harper extended his concept to include *Pyronema omphalodes* (*P. confluens*) of the Pezizales. Gwynne-Vaughan accepted Harper's concept of two nuclear fusions, one in the ascogonium and the other in the ascus, and expanded it by describing two reductional divisions in the ascus. The second reduction division, which was believed to occur in the various ascomycetes, either in the second or the third division in the ascus, was called "brachymeiosis". Thus the tetraploid number of chromosomes in the primary ascus nucleus was believed to be reduced to the haploid number by the end of the second or third division. This concept was developed by Gwynne-Vaughan and her followers primarily in their studies of the Pezizales. Many

investigators have disagreed with Gwynne-Vaughan, and several (Olive, 1949*b*, 1950; Hirsch, 1950; Wood, 1953) have recently shown, with the aid of the carmine or orcein smear techniques and with photographic evidence, that brachymeiosis does not occur in certain species in which it had been reported by Gwynne-Vaughan and her co-workers. Thus far, there has never been any genetic evidence demonstrating that brachymeiosis occurs in any species, but at the same time the process has not been genetically disproven in any species in which it was said to occur by the Gwynne-Vaughan group. The various genera of Pezizales included in the following discussion are arranged in the order of their occurrence in Seaver's monograph.

In his investigation of *Ascodesmis microscopica*, a homothallic species, Swingle (1934) observed that multinucleate antheridia and ascogonia ("oogonia") are produced in clusters on the same mycelium. Plasmogamy occurs when the trichogyne fuses with the antheridium. Swingle noted that the antheridial nuclei migrate into the ascogonium and pair with the ascogonial nuclei, following which the nuclear pairs fuse. Asci develop from ascogenous hyphae by crozier formation, and a second nuclear fusion was reported in the young ascus. It is interesting to note that Claussen had earlier described two nuclear fusions in the life cycle of this same species, but later reversed his opinion after he failed to find nuclear fusions in the ascogonium of *Pyronema omphalodes*.

Swingle's account of sexual reproduction in *A. microscopica* is similar, up to nuclear pairing in the ascogonium, to that described earlier by Schweizer (1931) in *Ascobolus strobilinus*, a homothallic species. The latter investigator found that the nuclei pass into the ascogenous hyphae in pairs without fusing, the only karyogamy occurring in the young ascus. In *A. stercorarius* Schweizer (1932) found that the asci are produced by croziers formed on ascogenous hyphae with binucleate cells. He observed that nuclear fusion occurs in the ascus, but not in the ascogonium as claimed by Welsford and others of the Gwynne-Vaughan school. Convincing evidence concerning the nature of plasmogamy in this heterothallic species is not available, although it appears likely that a distinct antheridium is not produced. Björling (1941) has claimed that the asci are not produced by means of croziers in *A. stercorarius*. He observed karyogamy in the young ascus. Nuclear fusions were

also reported to occur about the same time among some of the remaining nuclei in the ascogonium, as well as in vegetative cells of the apothecium, but these fusions may be a result of cytoplasmic degeneration. During prophase basophylic extranuclear bodies were seen in the cytoplasm, while within the nucleus the chromosomes condensed into small bodies and the nucleolus broke up into two or three smaller masses. Chiasmata were observed during diakinesis. The bivalent nucleolus chromosome is apparently attached to the nucleolus by means of two paired satellites. After the first division only one satellite is associated with each reorganized nucleolus. These observations support the contention that only one nuclear fusion occurs in the life cycle. However, Björling reported 16 bivalents in the fusion nucleus (8 having been previously reported by Fraser and Brooks, in the closely related *A. furfuraceus*) and he has discussed the likelihood that this is due to polyploidy. This seems unlikely in view of the fact that the asci are no larger than is normal for the species. In our own laboratory we have examined the asci of *A. stercorarius* with the aid of the carmine technique and have obtained evidence (unpublished) that there are only 8 bivalents, some of which are strongly heteropycnotic and at first give the impression of being many small chromosomes. We have also found that croziers are produced in this species.

Björling (1944) has claimed that *A. equinus* ($n = 4$–5?) is apomictic and that the asci develop by haploid parthenogenesis. A single haploid nucleus in the ascus reportedly divides three times successively to produce the eight ascospore nuclei.

The first clear-cut example of heterothallism in the ascomycetes was reported in 1920 by Dodge in *Ascobolus magnificus*. Later workers confirmed his findings. Gwynne-Vaughan and Williamson (1932) were the first to study *A. magnificus* cytologically. They found that each strain produces both male and female sex organs when two compatible strains are mated. Both the antheridium and the ascogonium contain large numbers of nuclei, and plasmogamy is accomplished with the aid of a trichogyne. Antheridial nuclei migrate into the ascogonium and pair with the nuclei of the latter. Gwynne-Vaughan and Williamson claimed that these nuclei fuse in pairs in the ascogonium. Diploid nuclei were then believed to pair in the ascogenous hyphae, the asci being

produced by crozier formation, and another nuclear fusion was reported in the ascus. Thus, the primary ascus nucleus was believed to be tetraploid. These investigators further stated that there are 8 bivalents in this nucleus and that 8 chromosomes pass to each pole in the first division, 8 in the second, 4 in the third (brachymeiosis). Thus, the haploid chromosome number was thought to be 4. It was also claimed that 4 chromosomes pass to each pole during nuclear divisions in the ascogonium, whereas 8 were observed during mitosis in the ascogenous hyphae. Wood (1953) studied *A. magnificus* with the carmine technique and found no evidence of brachymeiosis. He observed that 8 chromosomes pass to each spindle pole in all three divisions in the ascus. One of the longer chromosomes, though not the longest, is the nucleolus organizer. The deeply staining bodies in the nuclei by means of which Gwynne-Vaughan reported that she was able to distinguish between "male" and "female" nuclei were thought by Wood to be heterochromatic regions of the chromosomes. He observed them in the fusion nucleus as well as in the nuclei of the ascospores.

A species of *Aleuria*, related to *A. wisconensis*, was investigated by Aldinger (1936) who claimed that he obtained some evidence of nuclear fusion in the ascogonium. Karyogamy in the ascus is followed by three successive nuclear divisions, and the first and third divisions were considered reductional. Eight bivalents were seen on the metaphase spindle of the first division, while 4 chromosomes were reported at this stage of the third division. Some of the illustrations, however, indicate that more than 4 chromosomes pass to each pole in the last division, and it appears likely that the haploid chromosome number is 8. The ascospores are delimited by the centrosome-astral ray process.

The description of meiosis in *A. rutilans* by Wilson (1937) is one of the most detailed and probably one of the most accurate accounts which have appeared in the study of fungus cytology (Figures 56–58). The staining techniques used by most earlier investigators, that is, iron-alum haematoxylin and gentian violet, as well as the Feulgen technique, were used in this study. The various meiotic prophase stages, including pairing of the sinuous beaded chromosomes, appearance of the four-strand stage with chiasmata at diplotene and diakinesis, and the location of the

centromeres have been carefully described. Actual observations of the four-strand stage are very rare in cytological reports on the fungi. Probably the unusually large size of the chromosomes in *A. rutilans* has facilitated such observations here. In late prophase an intranuclear centrosome was found to divide, the developing spindle cones shifting into line as the centrosomes move to opposite sides of the nucleus. Sixteen chromosomes pass to each pole, during which their double nature is frequently apparent. At anaphase of the following two divisions, 16 chromosomes pass to each pole. Even at prophase of the third division, each chromosome was observed to contain two spirally coiled chromonemata. During these divisions the nucleolus disappears, and a new one is formed in each new nucleus. At the end of the third division the ascospores are delimited by the centrosome-astral ray process. Wilson also observed dividing nuclei in the croziers and found that 16 chromosomes pass to each pole. These observations are in agreement with an earlier report by Guilliermond that the haploid chromosome number is 16, but they tend to refute the account given by Gwynne-Vaughan (Fraser) that two nuclear fusions and brachymeiosis occur in the life cycle.

Probably no other discomycete has had so many cytological reports concerning its life history as has *Pyronema omphalodes*, (*P. confluens*), a homothallic species. Harper first studied the fungus cytologically and in 1900 reported that, following plasmogamy, the antheridial and ascogonial nuclei fuse in pairs in the ascogonium. Dangeard, however, believed that the antheridial nuclei degenerate, while Claussen reported that the antheridial nuclei pair with the ascogonial nuclei, and both believed that karyogamy occurs only in the ascus. W. H. Brown described a variety of this fungus in which plasmogamy fails to take place, and his findings are more in line with the report of Dangeard. In view of these and more recent investigations, it seems likely that *Pyronema omphalodes* may develop by plasmogamy or by haploid apogamy. More recently Moreau and Moreau (1930) stated that, although multinucleate antheridia and ascogonia with trichogynes appear and the trichogyne fuses with the antheridium, the antheridial nuclei degenerate without migrating into the ascogonium. The ascogonial nuclei were found to pair among themselves. These investigators (1931) reported that 12 chromosomes, the haploid number, pass

to each spindle pole in all three divisions in the ascus. Gwynne-Vaughan and Williamson (1931) have supported Harper's contention that plasmogamy is followed by fusion of antheridial and ascogonial nuclei in the ascogonium and by another fusion in the ascus. They claimed that 12 bivalents are present at meiotic prophase and that brachymeiosis occurs in the second division, 6 chromosomes going to each pole during this and the third division. Harper reported 10 as the haploid number, Dangeard 4, and Claussen 12. Raymond (1933*b*) believed that the number was 4, although he observed other chromatic bodies which he was unable to identify. He concluded that karyogamy occurs only in the ascus. Hirsch (1950), using the carmine technique, studied the asci of *P. omphalodes* and found that about 12 chromosomes pass to each pole in the first division as well as in the third division. Although her photographs are not definitive enough to show the exact number of chromosomes, it is evident that there are more than 6 (as reported by Gwynne-Vaughan and Williamson) passing to each pole in the third division and that brachymeiosis does not occur. Wilson (1952), in the latest study of this species, observed that the nuclei in the ascogenous hyphae are arranged in single file and divide in that position simultaneously. She has shown by means of diagrams how unlike nuclei might become included in the resultant binucleate cells, provided the ascogenous hyphae in such cases are heterokaryotic. The haploid chromosome number observed in the nuclei of the ascogenous hyphae was found to be 12, and 12 bivalents were observed in the diploid fusion nucleus. Cytological studies by individual investigators of isolates of such fungi as *P. omphalodes* from various localities would be a distinct contribution, as there is a possibility that these varied chromosome counts are explicable on the basis of heteroploidy.

According to Gwynne-Vaughan and Williamson (1930), no antheridium appears in the heterothallic *Ascophanus granulatus*, but the mycelium of either compatibility type is able to produce ascogonia when cultures are paired. The report of anastomoses between "plus" and "minus" mycelia is somewhat unusual. Nuclear fusions were described in the ascogonium as well as in the asci. The chromosome number is reportedly reduced from 16 to 8 in the first nuclear division and from 8 to 4 in the second division (brachymeiosis), with 4 also passing to each pole in the third

division. The observation of 4 chromosomes passing to each spindle pole during division of the nuclei (supposedly diploid) in the ascogenous hyphae is explained on the basis of a premature association of allelomorphs. These same investigators (1934) also studied *A. aurora*, where plasmogamy and nuclear fusion in the ascogonium, as well as in the asci, were reported. Four bivalents were found at meiotic prophase, but only 2 chromosomes were found to pass to each pole in the third, or brachymeiotic, division.

The cytological details of ascus development described by Eftimiu (1929*c*) in *Patella* (apparently *P. scutellata*) are similar in all essential respects to those which he reported for *Geoglossum hirsutum* and *Spathularia flavida*, which have already been discussed. The haploid chromosome number is 4. There was no evidence of brachymeiosis. Raymond (1934) obtained similar results with *Patella stercorea*, in which it was also found that the haploid chromosome number is 4. On the other hand, Gwynne-Vaughan and Williamson (1933) reported that brachymeiosis occurs in the second nuclear division in the ascus of *P. scutellata*. Twelve bivalents were described at meiotic prophase, and each ascospore was found to receive 6 chromosomes, one long and five short ones. In the heterothallic species, *P. melaloma*, Gwynne-Vaughan (1937) observed that a coiled ascogonium with functionless trichogyne appears, while the antheridium is lacking. Apparently, plasmogamy occurs by means of hyphal fusions at the base of the ascogonium. Nuclear fusions were reported in the ascogonium. At meiotic prophase 4 bivalents appear, and 4 chromosomes pass to each pole in the first and second divisions. Brachymeiosis was believed to occur in the third division, with 2 chromosomes going to each pole. Olive (1949*b*, 1950) reinvestigated *P. melaloma* with the iron alum-carmine technique and found that in all three divisions 4 chromosomes—one long (the nucleolus organizer), two slightly shorter, and one very short—pass to each pole. The latter findings were substantiated with photographic evidence.

Nardi (1930) found that the asci of *Peziza praetervisa* arise from terminal binucleate cells in the hymenium, rather than from croziers. The account of the chromosomes being formed by aggregations of smaller "protochromosomes" can not be seriously considered. It seems more likely that the latter are actually

heterochromatic regions (chromocenters) of the chromosomes. Pinto-Lopes (1949) has also expressed the opinion that the "protochromosomes" of various authors are chromocenters. The spindle of *P. praetervisa* originates in the same manner as described by Wilson (1937) for *Aleuria rutilans*. In all three nuclear divisions in the ascus, 8 chromosomes were believed to pass to each spindle pole. The developments in the ascus of *Plectania coccinea* are similar, and the chromosome number is probably the same. In *Lamprospora leiocarpa* the haploid number is 4. Valkanov (1931) studied the asci of an unidentified *Peziza*-like fungus in which he found polar caps, apparently of intranuclear origin. These are disc-shaped chromatic structures that appear at each end of the spindle. It is not certain that they are centrosomal in nature. The Moreaus (1931) reported a haploid number of 8 chromosomes in *Peziza vesiculosa*, with no evidence of brachymeiosis. Raymond (1934) found that $n = 4$ in *P. succosa*. Matsuura and Gondo (1935) reported 4 bivalents in the primary ascus nucleus of *P. subumbrina* and found that 4 chromosomes pass to each pole in all three divisions in the ascus. They claimed that one dissociated pair of heteromorphic chromosomes is present and may represent sex chromosomes. However, the cytological evidence presented is not conclusive, particularly in view of the fact that a number of the drawings and photographs strongly indicate that the haploid chromosome number is greater than 4. The assumption that these larger numbers may be explained on the basis of a precocious splitting apart of the chromosomes has never received adequate confirmation in any definitive cytological work on the fungi.

Karyogamy and meiosis were observed in *Morchella deliciosa* by Wakayama (1930*b*) who described a synizetic phase ("synaptic knot") during meiotic prophase and at prophase of the third nuclear division in the ascus. Twelve chromosomes pass to each pole of the first-division spindle which is provided with centrosomes and astral rays, the chromosome number thus being reduced from 24 to 12. Twelve chromosomes also pass to each pole in the second division, but in the third division only 6 were observed. Six were also found during mitosis in the ascospores. The latter are delimited by astral rays emanating from beaked centrosomes. Although the results appear to support the theory of brachymeiosis,

Wakayama reserved final opinion on this until further studies could be carried out. It is interesting to note that some tetraploid asci were found with 48 chromosomes, 24 passing to each pole at anaphase of the first division. In a cytological study of several species of *Morchella*, Greis (1940) found that the dikaryophase in *M. conica* and *M. esculenta* arises by the fusion of two hyphae at their tips in the hymenium or sub-hymenium. The ascogenous hyphae with paired nuclei which develop from these fusion cells give rise to the asci. Karyogamy occurs only in the ascus. In *M. elata* there is no evidence of plasmogamy, and a single terminal or intercalary cell gives rise to the ascogenous hypha. Here, too, the nuclei become arranged in pairs. In none of the species was crozier formation observed.

TUBERALES. These hypogeous ascomycetes, commonly known as truffles, have been greatly neglected in cytological work, and little is known concerning their nuclear behavior. In a series of papers on the morphology and cytology of *Tuber aestivum* and *T. brumale*, Greis (1939*a*, 1938*b*, 1939) has reported that no recognizable sex organs are present and that the dikaryophase arises in the young fructification by a fusion of two uninucleate hyphal tips, as in *Morchella*. The fusion cell gives rise to an ascogenous hypha in which the paired nuclei divide conjugately. This hypha eventually becomes divided into binucleate cells with clamp connections at the cross walls. The terminal cells as well as some of the intercalary cells give rise to the asci by means of crozier formation. Greis (1938*a*) compared the ascogenous hyphae to the dikaryotic mycelium of basidiomycetes and emphasized their phylogenetic importance. In his opinion, the clamp connections in most basidiomycetes are functionless croziers which earlier had the function of increasing the number of zygotes. He has pointed out that this function still persists in a number of basidiomycetes where clusters of clamps increase the number of basidia. The fusion nucleus in the ascus of *Tuber* and the vegetative nuclei were found to have a distinct nucleolus and one or more smaller chromatic bodies which were not identified. In view of several recent cytological studies which have already been discussed, such bodies might be considered as heterochromatic. In the first meiotic division, particularly in *T. aestivum*, Greis noted that the nucleolus sometimes becomes dumbbell-shaped and constricts into two parts, one going

into each new nucleus. The evidence is not sufficient to show that this is the normal procedure, however. Indications of irregular nucleolar division were also observed in later mitoses. Only two nuclear divisions were reported to occur in the ascus prior to spore formation. All four nuclei may persist, or from one to three may degenerate. Therefore the number of ascospores in the different asci varies from one to four.

BASIDIOMYCETES

The most distinctive single structure in this class of fungi is the basidium which, in the opinion of most students of the fungi, has been phylogenetically derived from the ascus. Like the ascus, it is the cell in which karyogamy (with certain exceptions) and meiosis occur. However, its meiospores, typically four in number, are produced externally instead of internally as in the ascomycetes. The orientation of the spindles during meiosis has been used as a primary diagnostic character in the taxonomy of basidiomycetes by Bessey (1950) and certain others, whereas Rogers (1934) has pointed out the inadequacy of this criterion in maintaining a stichobasidial series (spindles vertically oriented) and a chiastobasidial series (spindles transversely oriented). An examination of the accumulated cytological data on this subject indicates that spindle orientation may, within certain limits, be of some taxonomic value, although it seems unlikely that it can be used, at least in the homobasidiomycetes, as the primary criterion. Generally, it is believed that when the basidium is broad, the spindles tend to be transverse, whereas when the basidium is long and narrow, they are vertical. Frequently, in the same species the spindles may be found to range in orientation from vertical to transverse or oblique.

Although there are many variations among the basidiomycetes with regard to the life cycle, there are certain features common to the majority of them. In most cases the basidiospore is homokaryotic and gives rise to a septate mycelium with a single nucleus (sometimes several) in each cell. In the majority of species which have been studied, it is necessary that two compatible monokaryotic mycelia be brought together before the life cycle can be completed. Such species are heterothallic. As has been mentioned previously, Whitehouse (1949*b*) concluded that the rusts and smuts are characterized by a type of heterothallism, called bipolar heterothallism,

involving only two allelomorphic factors, whereas the Tremellales, hymenomycetes, and gasteromycetes are characterized by both bipolar and tetrapolar heterothallism with multiple allelomorphs at the loci for compatibility. A minority of the basidiomycetes are homothallic.

With the possible exception of the rusts, there is no semblance of sex organs in the basidiomycetes. Nevertheless, the salient features of sexual reproduction—karyogamy and meiosis—are present. In heterothallic species the dikaryon typically arises by means of anastomoses between hyphae, between oidia or between oidia and hyphae of compatible strains. The dikaryophase of the basidiomycetes, unlike that of the ascomycetes, is an independent mycelium. It is generally composed of binucleate cells with clamp connections, and its probable phylogenetic relationship to the ascogenous hyphae has already been discussed. The dikaryophase eventually gives rise to the fruiting body, on which the basidia are produced. The only truly diploid cell in the life cycle is the basidium with its fusion nucleus. A diagrammatic representation of a typical heterothallic life cycle is presented in Figure 60.

A homothallic species is able to complete its life cycle when grown from a single haploid basidiospore. A dikaryophase, in which genetically similar nuclei are paired, appears in the mycelium, or occasionally in the hymenium only. Apparently, a distinguishing terminology is needed here, as there are obviously two types of dikaryons in both ascomycetes and basidiomycetes. It is suggested that wherever the distinction is needed, a dikaryon composed of genetically similar nuclei be referred to as a " homodikaryon " and that a dikaryon composed of genetically dissimilar nuclei be called a " heterodikaryon ". In homothallic basidiomycetes, as in heterothallic ones, karyogamy and meiosis occur in the basidium.

Although the homothallic type of life cycle has been considered inferior by many investigators, the same advantages probably exist here as have already been discussed in connection with the Aspergillaceae and Helotiaceae of the Ascomycetes. Although the information on this subject is too scant to allow any very definite statements, it is to be expected that heterokaryosis, followed by hybridization and segregation in the basidium, may occur in homothallic basidiomycetes when mycelia differing in phenotype or biotype are brought together. At least one investigator (Kligman,

1943) has obtained some evidence which indicates that this is possible. The prediction may be made that heterokaryosis, heterozygosity and segregation will be found to occur quite commonly among homothallic fungi in general. Whitehouse (1949*a*) has prepared a list of the various species of homobasidiomycetes and jelly fungi in which homothallism and heterothallism (bipolar and tetrapolar) have been determined.

In the treatment that follows, the heterobasidiomycetes, which typically have septate basidia, are discussed under four main orders, while the homobasidiomycetes, which have non-septate basidia, are discussed under the two main groups—hymenomycetes and gasteromycetes.

UREDINALES. Although a few of the rusts have been reported to be homothallic, the great majority of species studied have proved to be heterothallic. In most cases compatibility seems to be controlled by a single pair of factors. In a typical long-cycle heteroecious rust, such as *Puccinia graminis*, the dikaryophase originates on the barberry, two compatible strains of the rust being required for its initiation, and binucleate aeciospores from aecia produced on the barberry infect the wheat plant. The dikaryotic mycelium produced in the wheat gives rise to binucleate uredospores, which reinfect the wheat, and later to two-celled teliospores in which karyogamy occurs. After the teliospore has overwintered, each of its two cells produces a basidium in which two successive meiotic divisions occur, and the basidium, now four-celled, gives rise to haploid basidiospores which infect the barberry. The dikaryophase in rusts is devoid of clamp connections. The discovery by Craigie (1927, 1933) that the pycniospores of the rusts can initiate the dikaryophase by fusing with a flexuous hypha in the pycnium of opposite compatibility type has done much to stimulate investigations on the cytology and genetics of these organisms. The recent report by Hotson and Cutter (1951) concerning the culture of rusts on artificial media will undoubtedly bring about renewed interest in the study of this group.

Some of the publications which review the cytology and genetics of rusts are those of Arthur (1929), Dodge (1929), Buchwald (1933), Allen (1933), Lamb (1935), Craigie (1942) and Stakman (1947).

The numerous papers of Allen have contributed much to our knowledge of the morphology and cytology of the rust fungi. The

various methods by which the heterokaryotic condition is initiated are summarized by her (1934*b*). They include (*a*) anastomoses between hyphae of compatible mycelia, (*b*) fusion of compatible pycniospores with receptive hyphae in the pycnium, (*c*) fusion of pycniospores with stomatal hyphae, (*d*) germination of the pycniospore and entrance of the germ tube through a stoma " to become effective ", (*e*) fusion of pycniospores in pairs (Allen, 1930). Conclusive evidence in support of the last three methods is yet needed, but most investigators are probably in agreement that the first two methods are common, although Rice (1933), in a study of four rusts, reported that she failed to find evidence of fusions between pycniospores and receptive hyphae in any species. In flax rust, according to Allen (1934*a*), pycniospores may infect the host in the vicinity of compatible mycelia and actually give rise to mycelia in the host tissue, with binucleate cells eventually arising through cell fusions in the aecial primordium.

Allen (1929) reported that, even in the absence of the alternate host and therefore of the haplophase of *Puccinia triticina* (*P. rubigo-vera*), there are possibilities for the occurrence of new combinations of nuclei in the hyphae. She found that there are occasional fusions of two appresoria or of two sub-stomatal vesicles of germinating uredospores. Also occasional anastomoses between hyphae in regions of developing uredinia were reported. Brown (1932), repeating with *P. helianthi* an experiment somewhat similar to that described by Buller (1930) for *Coprinus lagopus*, found that when uredospores of the rust were sown around the pycnia of single-basidiospore infections, the heterokaryotic condition was apparently initiated in the monokaryon mycelium, and eventually normal aecia were produced on the underside of the infected leaves.

Andrus (1931, 1933), in a study of *Uromyces appendiculatus*, believed that he had found a method of sexual reproduction similar to that reported in the red algae. He claimed that the so-called " two-legged cells " in the aecial primordium, described by others as binucleate cells resulting from cell fusion, are in actuality uninucleate at origin. A hypha or " trichogyne " was believed to lead from this cell, which he called the " egg " cell, to the surface of the leaf where it serves as a receptive hypha. Pycniospores were thought to fuse with this hypha, after which a male nucleus migrated into the egg cell, thus " fertilizing " it. Accessory cell fusions were found to occur later in the young aecium Although

certain features of Andrus's work are in keeping with what has been found in other rusts, this interpretation of aecial ontogeny has not received general acceptance.

Lamb (1935) found that the dikaryophase in the macrocyclic *Puccinia phragmitis* arises in the aecial primordium by means of cell fusions or by fusions of pycniospores with receptive hyphae. It is interesting to note that Lamb reported multinucleate cells and cell fusions in single-spore infections, although aeciospores were not produced. Wang and Martens (1939), after having studied four long-cycle rusts in addition to the short-cycle *P. malvacearum*, denied the occurrence of lateral cell fusions (" Christman fusions ") in the aecial primordium (or telial sorus, in the case of *P. malvacearum*). They believed that the aecial primordium is derived from binucleate hyphae which originate outside the primordium by means of hyphal fusions or fusions between pycniospores and receptive hyphae. Their report that the pycniospores of *P. poarum* may conjugate on the leaf surface and possibly may even give rise to infection hyphae is in need of further study. In *Thekopsora hydrangeae* Olive (1943*b*) found no evidence of a stable binucleate condition in the hyphae outside the aecial primordium, although hyphal fusions with accompanying nuclear migrations were frequently encountered in this area. The first permanently binucleate cells appeared following lateral cell fusions in the aecial primordium.

In the microcyclic rust, *Puccinia prostii*, Lamb (1934) found that the dikaryotic condition arises at the base of the telial primordium, either by means of cell fusions or by nuclear migrations. The species was suspected of being heterothallic. Burges (1935) observed that the dikaryophase in *Uromycladium tepperianum* arises by means of cell fusions at the base of the young telium.

As has already been mentioned, most rust fungi are heterothallic, and the studies of Allen and others indicate that a single pair of allelomorphs is involved in each species. However, according to Brown (1940), at least four species of *Puccinia* are homothallic. Three of these—*P. malvacearum*, *P. grindeliae*, *P. xanthii*—are microcyclic species, while the fourth species, *P. coronata eleagni*, is macrocyclic and heteroecious. The evidence for homothallism is quite convincing in the microcyclic species but less so in *P. coronata eleagni*, in which case fewer single-basidiospore infections were

studied and in which these infections generally required an unusually long period of time for aecial production. In all four species Brown reported that the binucleate stage (homodikaryon) arises by mitotic division of the nucleus in cells of the primordium without an accompanying cell division Until Brown's publication appeared, homothallism had been claimed only in *P. malvacearum*. Ashworth (1931) reported that single-basidiospore infections gave rise to the telial stage, but the percentage of infections reaching this stage was found to be very low. Allen (1933, 1935) later studied the same species, concluding that it is not homothallic and that paired monosporous infections are necessary for the production of telia. Her report of 100 per cent compatibility among paired single-spore infections is difficult to understand.

As in most other fungi, the nuclei in the vegetative hyphae of rusts are relatively small, and most of the information concerning nuclear structure and the details of nuclear division is based mainly on studies of the diploid nucleus and meiosis. The fusion nucleus is in many cases quite large, and it is doubtful that the fusion nucleus in any other group of basidiomycetes attains the size and clarity of detail found in the rusts. In *Coleosporium* the diploid nucleus reaches a diameter of 13 μ (Olive, 1949*a*), and in *Gymnosporangium* it attains a diameter of 10 μ (Berliner, 1953).

Detailed studies of karyogamy and meiosis in the rusts show that these processes are very similar to those in most other fungi (Figures 61–74). In *Puccinia malvacearum* Allen (1933) found that karyogamy occurs after cross-wall formation in the teliospore while the nuclei are still quite small. Details of synapsis are obscured in such small nuclei, but nucleolar fusion was observed. Similar developments were reported in *Gymnosporangium* by Stevens (1930). Ashworth (1934) described karyogamy and meiosis in *Coleosporium tussilaginis* and reported that the haploid chromosome number is 8 to 10. In *Coleosporium*, Olive (1942, 1949*a*) and Sanwal (1953) found that the fusing nuclei were sufficiently large to permit more detailed study of synapsis. Very early in the process of nuclear fusion, the chromosomes of the two haploid sets come together and pair, the two conspicuous nucleoli being brought together into one by synapsis of the nucleolus chromosomes. Following synapsis there is a "phase of elongation" during which the chromosomes become much extended and narrow,

as the diploid nucleus enlarges. This coincides with enlargement of the probasidium and probably represents a metabolic phase. The nucleolus disappears during diplotene, and at diakinesis 8 shortened bivalent chromosomes appear in the nuclear vacuole. In the two successive meiotic divisions which follow, 8 chromosomes pass to each spindle pole. The spindles, which are provided with centrosome-like bodies with asters, may be oriented in various directions in the basidium. In an excellent publication by Holden and Harper, which appeared much earlier, similar spindles with 6 to 10 chromosomes passing to each pole were described. The cross walls, which are apparently delimited with the aid of the recurving astral rays, always develop at right angles to the long axis of the spindles. A third nuclear division takes place in the basidiospore. Both Olive and Sanwal have stressed the possible phylogenetic significance of these developments in connection with the theory that the rusts have been derived from ascomycetous ancestors. The cytology of *Ochropsora sorbi*, which has basidia somewhat similar to those of *Coleosporium*, has been described by Soong (1939) who believed the haploid chromosome number to be 10 or more. There was no evidence that astral rays assist in cross-wall formation.

Allen (1933) observed spindles with centrosome-like bodies in *Puccinia malvacearum*. She believed that the haploid chromosome number is 5. Stevens (1930) reported that the haploid number in *Gymnosporangium juniperi-virginianae* is 2, although some of her illustrations indicate the presence of larger numbers. Berliner and Olive (1953) and Berliner (1953) studied several species of *Gymnosporangium* with the aid of a specially developed iron alum-carmine technique and found the haploid chromosome number to be 8. Information on the size and morphology of the chromosomes was also obtained. These authors further described the interesting effects of antibiotics such as streptomycin and actidione upon meiosis in *Gymnosporangium*. Such phenomena as lagging of chromosomes and the loss of certain chromosomes from the spindle, uneven grouping of chromosomes at telophase, and eccentricites in cross-wall formation in relation to nuclear divisions were reported.

As the present reviewer (1942) has previously pointed out, many of the earlier, as well as recent, reports of 2 chromosomes as

the haploid number in fungi must be considered with some skepticism. During anaphase the spindle fibers show a frequent tendency to come together into two main strands along which the chromosomes pass to the poles. Whether this is due to the method of fixation is not known. The present writer has observed it in material fixed in a number of the more commonly used solutions, but to a much less extent in material prepared by means of the iron alum-carmine technique. The result is that at telophase the chromosomes tend to clump at each pole into two groups, mistaken by many investigators as 2 chromosomes. This is not to say that no fungi have a haploid number of 2, as the detailed investigations of some workers (e.g., Wakayama, 1930*a*, 1932) indicate that some species may have this number.

Pady (1934, 1935, 1946) has studied karyogamy and meiosis in several of the Melampsoraceae. In *Hyalopsora aspidiotus* he observed that the fusion nucleus may pass into the later stages of prophase, occasionally as far as diplotene, before entering into the overwintering resting stage. Olive (1943*b*) studied the cytological developments throughout the life history of *Thekopsora hydrangeae*, another member of this family, in which he haploid chromosome number was believed to be 4.

In a controversial publication on the structure and behavior of the nuclei in rusts, Savile (1939) emphasized that there are two structural types of nuclei, found at different stages in the life cycle. The " unexpanded " nucleus was believed to be present in all phases of the life history where nuclear migration is necessary, while the " expanded " nucleus was reported in aeciospores, uredospores, teliospores, and in the basal cells and spore-mother-cells. Savile also believed that the body considered to be a nucleolus by most investigators is not a true nucleolus, but is composed mostly of chromatin and may have a true nucleolus within it. The studies of Olive (1942, 1949*a*) and Sanwal (1953) strongly indicate that Savile's concept of two types of nuclei is not acceptable, and that the nucleus in a vegetative hypha or the nucleus migrating through the narrow sterigma into the basidiospore has essentially the same structure as the fusion nucleus, except, of course, that the latter is diploid. Furthermore, the discovery that a typical nucleolus and nucleolus chromosome are present (Allen, 1933; Olive, 1942, 1949*a*) emphasizes that nuclear structure in the rusts is basically

like that of higher plants. Savile believed that the haploid chromosome number is 4 in *Puccinia malvacearum, P. sorghi, Uromyces fabae, U. hyperici* and *Melampsora biglowii.*

In a number of species of Uredinales certain cytological developments have been reported which are unknown in the majority of rusts. Some of the more unusual life cycles are reviewed by Dodge (1929). Certain species, e.g., *Kunkelia nitens* and *Endophyllum euphorbiae-sylvaticae,* have no nuclear fusion in their life cycle, although basidia are produced. No true teliospore is formed, and the basidium is produced by what appears to be an aeciospore. The two nuclei migrate into the basidium and divide once, after which cross walls partition the basidium into four uninucleate cells. One form of *Kunkelia nitens* exists entirely in the monokaryotic condition, the uninucleate aeciospore giving rise to a two-celled basidium following a single mitosis. The cytology of the two forms of *Kunkelia nitens* was studied earlier by Dodge.

Ashworth (1935) observed that the basidia of *Endophyllum sempervivi* are produced by cells resembling aeciospores. Karyogamy occurs in these spores, after which the fusion nucleus migrates into the developing basidium and undergoes meiosis. A four-celled basidium is produced. In *Cystopsora oleae* karyogamy occurs in the teliospore, and the latter germinates to produce a basidium with two binucleate cells or a normal basidium with four uninucleate cells (Thirumalachar, 1945). Lindfors had earlier described two-celled quadrinucleate basidia in *Puccinia arenariae.* He also found that a single basidiospore, upon infecting the host, produces a mycelium with binucleate cells. Since single-basidiospore infections were not studied in *C. oleae,* it is not known whether the fungus is homothallic or heterothallic. If basically heterothallic, as is probably the case, then the basidiospores of the two-celled basidia are most likely miktohaplontic. In *Uromyces aloes* (Thirumalachar, 1946) the fusion nucleus undergoes the usual two meiotic divisions, during which the haploid chromosome number was found to be 6, and a four-nucleate basidium is produced. A single cross wall generally divides the basidium into a lower uninucleate cell and an upper three-nucleate cell. No basidiospores develop, but an infection hypha is produced by the three-nucleate cell. It is quite likely that this species is basically heterothallic and that the infection cell is miktohaplontic.

In *Sphenospora kevorkianii* the fusion nucleus undergoes two successive divisions in the basidium, but in most cases only two septa are formed, and the basidium becomes divided into one binucleate and two uninucleate cells (Olive, 1947). Most often it is the middle cell which is binucleate. Occasionally, two- and four-celled basidia are produced. If this rust should prove to be heterothallic, then, again, it would appear likely that the binucleate basidial cells and the basidiospores produced by them are miktohaplontic. In *Gymnosporangium clavipes* (Olive, 1949*c*) it has been found that a small percentage of the basidia are three-celled, with one of the cells—usually the middle one—binucleate. This rust is undoubtedly heterothallic, and it seems quite probable that the basidiospores produced by the binucleate basidial cells are in most cases heterokaryotic for the compatibility factors, in which case single-spore infections of the alternate host by these basidiospores would be expected to give rise to aecia. The discovery of such basidiospores in *G. clavipes* indicates that a similar condition may exist in other rusts which normally have four-celled basidia. This may also explain reports by a number of investigators that occasional single-basidiospore infections in these rusts give rise to aecia.

The studies of Stakman et al. (1930), Newton et al. (1930), Johnson and Newton (1940*a*, 1940*b*, 1943), Flor (1942) and others concerning physiologic races and the inheritance of pathogenicity and other characters in the rust fungi will be familiar to many readers. The important features of the work on hybridization in rusts and smuts and the inheritance of factors for pathogenicity have recently been reviewed in a very readable manner by Stakman (1947). No attempt is made to review these investigations here. However, one feature of the work which appears to be particularly applicable to the present discussion will be considered. Newton et al. (1930) and Johnson and Newton (1940*b*) studied the inheritance of two pairs of factors determining spore color. They found that various combinations of these genes gave the same effects in the uredospores, where two haploid nuclei are paired, as in the teliospores, where the two nuclei had fused to form a single diploid nucleus before the color characters were expressed. These investigators very logically argued that dominance and recessiveness in this rust may be expressed as well in the dikaryophase as in the diplophase, thus opposing Dodge's assertion

that these phenomena can pertain only to the diplophase. Dodge coined the term "heterokaryotic vigor" to describe the growth rate obtained by deriving heterokaryons from two slow-growing mutants of *Neurospora tetrasperma*. Obviously, as in these rusts, not all heterokaryotic effects have to do with more vigorous growth, and other terms would need to be coined in such cases if strict homologies are to be observed. Actually, there would appear to be no valid reason for not referring to certain dikaryotic effects in terms of dominance and recessiveness, although adoption of this viewpoint would not justify homologizing the dikaryophase with the diplophase. Furthermore, it would be hazardous to apply this terminology to heterokaryotic mycelia in which the nuclei are not distinctly paired, since the ratio between the different genotypes in such mycelia undoubtedly would be variable.

USTILAGINALES. Smuts are characterized by the production of thick-walled sclerobasidia called "brand spores" or "smut spores". These are comparable to the teliospores of rusts, in that karyogamy occurs in them and the basidia are produced by them. In the Ustilaginaceae the basidium is typically four-celled and four-nucleate. In contrast to the condition in rusts and in other basidiomycetes, the nucleus in each cell remains there and divides mitotically to produce nuclei for the basidiospores or sporidia. Several basidiospores may be produced by each cell. The basidiospores are able to bud in yeast-like fashion. In the Tilletiaceae the basidium is generally non-septate, and four, eight, 16 or even more elongate basidiospores are produced apically on the basidium. There are many different methods by which the dikaryophase may arise in the smut fungi; these include fusions outside the host plant between basidiospores or between conidia produced by them, or between basidiospore or conidium and a basidial cell, or two basidial cells may copulate. Also, the dikaryophase may arise in some smuts by hyphal fusions within or outside the host tissue. Probably the most frequently reported method is the copulation of basidiospores or conidia. Boss (1927), however, has suggested that this method has been over-emphasized as a sexual process. For example, he observed that in *Ustilago violacea* triple and quadruple sporidial fusions may occur in addition to fusions in pairs, and there is no nuclear migration from one cell to the other. Furthermore, on nutrient media each cell of a fusion pair or group is still capable of producing uninucleate conidia. In *Tilletia tritici* the binucleate

secondary sporidia may give rise to similar but uninucleate conidia. Boss reported that there is no sporidial copulation in *Ustilago ischaemi* and that karyogamy fails to occur. Such a life cycle as this would be comparable to that of certain gill fungi which pass their entire life cycle in the monokaryotic condition.

There has been considerable debate about whether the dikaryotic mycelium of smuts possesses clamp connections. Seyfert (1927) studied a considerable number of species belonging to both the Ustilaginaceae and Tilletiaceae and reported that all of them, including the much studied *Ustilago zeae*, have clamp connections. The presence of clamp connections on the dikaryotic mycelium of *U. zeae* was confirmed by Stakman and Christensen (1927), who pointed out that they are absent on the monokaryon mycelium, and by Hanna (1929). On the other hand, Sleumer (1932) considered these structures as abortive branches instead of clamp connections. The report by Stempell (1935) that typical clamp connections are produced by certain mycelia in *Entyloma* has recently been placed in doubt by reports that his material was contaminated with *Itersonilia* (Derx, 1948; Olive, 1952). Further studies on the occurrence of clamp connections in the smuts are much needed.

A most peculiar method of maintaining the dikaryon has been reported in cultures of *Ustilago nuda* by Thren (1941). The two paired nuclei near the end of a hypha divide simultaneously, with the two spindles in tandem arrangement, and cross walls cut out a middle binucleate cell with a uninucleate cell on each side. The two uninucleate cells copulate by a hypha, while the middle dikaryotic cell branches laterally. The process is compared to crozier development and clamp formation, and the basic similarities are indeed striking.

In contrast to the condition in rust fungi, the fusion nucleus in the smuts is quite small, usually around 3 μ in diameter. Of course, the nuclei in the vegetative hyphae are even smaller. Cytological studies by D. T. Wang (1934) and others have shown that these nuclei are typical in structure. However, the study of nuclear structure and behavior in these organisms has, in general, been unrewarding, and there is still considerable doubt that anyone has been able to identify accurately the chromosomes in any species. Most of those who have attempted to describe meiotic divisions in the smuts have presented illustrations that are difficult to interpret.

Although Harper in 1899 described nuclear divisions in the

basidia of *Ustilago scabiosae* and found 8 to 10 chromosomes, most later investigators have reported much smaller numbers. Kharbush (1927*b*, 1928), Hüttig (1931), D. T. Wang (1932*a*, 1932*b*, 1934), C. S. Wang (1943) and Vaheeduddin (1942) have reported 2 as the haploid number for various species of Ustilaginaceae and Tilletiaceae, whereas Yen (1936) has reported 4 chromosomes (bivalents?) on the metaphase spindle in *Tilletia tritici*, and presumably 4 is the haploid number. Hirschhorn (1945), in a study of several species of Ustilaginaceae, described an unusual chromosomal behavior during the two divisions in the basidium. In the same species she found that 2, 3, or 4 chromosomes may pass to the spindle poles in the first or second division, and sometimes even in the following division. It is obvious from her illustrations and descriptions, however, that many of these irregularities resulted from the use of material inadequately fixed and stained. It seems likely that the details of meiosis in the Ustilaginales will not be convincingly worked out until more satisfactory staining techniques are devised.

One of the best reviews of the life cycles of the smut fungi, inclusive of cytological findings, up to the time of its publication is a paper by Sampson (1939). As she has observed, most of the life history studies have demonstrated that the smuts are predominantly heterothallic. There have been a few reports of homothallism, but probably in no case has true homothallism been proven. Homothallism has been claimed in *Urocystis cepulae* (Blizzard, 1926) and in *Ustilago striaeformis* f. *poae-pratensis* (Leach and Ryan, 1946), but in these species no sporidia are produced and the mycelium is derived from the germinating basidium, which most likely contains nuclei of both compatibility types. Apparently, the majority of investigators who have studied the inheritance of compatibility factors in the smut fungi are of the opinion that a single pair of factors is involved. There have also been reports of two pairs of factors, based upon crosses made in infection experiments (Hanna, 1929). Whitehouse (1949*b*) has pointed to possible errors in this technique and has claimed that the evidence for tetrapolarity in the Ustilaginales is inadequate. He stated that " in all the species investigated the heterothallism may have as its genetic basis one locus with two allelomorphs ".

The occurrence of " solopathogenic lines " in *Ustilago zeae* has been reported by Christensen (1932) and Chilton (1940, 1943).

In such cases single sporidial infections give rise to mycelia which produce normal brand spores. According to these investigators, there has probably been some interference with the normal disjunction of chromosomes in the basidium, and reduction is delayed until a later stage. Sporidia arising from such basidia are presumably diploid and heterozygous for the compatibility factors. Although the genetic evidence supports this concept, further cytological studies with improved staining techniques are needed to ascertain the chromosomal behavior.

SEPTOBASIDIALES. In his monograph of the genus *Septobasidium*, an exceptional group of heterobasidiomycetes living symbiotically with scale insects, Couch (1938) observed that the mycelium in *S. pseudopedicellatum* and *S. grandisporum* is composed of binucleate cells and that karyogamy precedes the nuclear divisions in the basidium, while in *S. jamaicense* the mycelium is composed of cells with one to several nuclei, a single nucleus being present in the young basidium and a single nuclear division preceding spore formation. In a study of basidial development in the genus, Olive (1943*a*) described six cytological types. In *S. alveolatum*, which is probably the most representative species, karyogamy takes place in the probasidium—an inflated persistent cell that gives rise to the basidium. Two meiotic divisions occur in the basidium, and the latter becomes transversely divided into four uninucleate cells, as in the Uredinales. Although the basidiospores are originally uninucleate, they become several-celled after mitosis. Development of the probasidium is the same in *S. sinuosum* and *S. grandisporum*. However, in *S. sinuosum* only one meiotic division generally occurs in the basidium, which becomes two-celled, and the second division apparently takes place in each of the two basidiospores before the latter are discharged. In *S. grandisporum* both meiotic divisions occur in the basidium but no cross walls are formed, and a single, large, multinucleate basidiospore is produced. Mitotic divisions continue to occur in the basidiospore, after which it becomes divided into many small cells. In *S. septobasidioides* karyogamy and meiosis takes place as in *S. alveolatum*, but no persistent probasidium is present. The same is true of *S. sinuosum*, except that the four-nucleate basidium becomes divided into three cells instead of four. One of these cells, usually the middle one, is binucleate, as in the rust *Sphenospora kevorkianii* (Olive, 1947). *S. jamaicense* has no persistent probasidial cell and the

young basidium is uninucleate at its origin. Therefore, karyogamy does not occur. The nucleus divides once and a two-celled basidium is formed. The two basidiospores are discharged in the uninucleate condition. Chromosomes could not be counted with certainty, but the haploid number for the various species was believed to be around 5. The report of nucleolar division in *Septobasidium* is apparently based on an error in observation and will probably be subject to a correction similar to that made for *Coleosporium* (Olive, 1949*a*).

Couch (1937) described a new genus and species of the Septobasidiales, *Uredinella coccidiophaga*, which he considered to be intermediate between the rusts and *Septobasidium*. Rust-like basidia are produced by thick-walled sclerobasidia that resemble teliospores, and binucleate spores comparable to uredospores are also present. Karyogamy occurs in the sclerobasidium, and two meiotic divisions take place in the basidium. There is evidence that the nucleus in each cell of the mature basidium remains in the cell and divides mitotically to produce the nucleus which passes into the basidiospore.

TREMELLALES. The jelly fungi are a heterogeneous group of heterobasidiomycetes, most of which are saprophytic. The mycelium producing the fruiting bodies may or may not be provided with clamp connections. The basidia in some groups are transversely septate; in others they are vertically septate, and in one large family they are non-septate. In Martin's system of classification, a number of families, often treated as orders by other authors, have been included within the single order. The basidial terminology used by Neuhoff, Martin and their students is not accepted in its entirety by the present reviewer, particularly with regard to the terms " hypobasidium " and " epibasidium " which do not consistently refer to homologous structures in the various families.

In the Auriculariaceae karyogamy occurs in the probasidium which may develop directly into the basidium or which may remain as a persistent empty structure at the base of the basidium. Typically, two meiotic divisions take place, and a transversely septate four-celled basidum is produced. In the genus *Helicogloea* (Baker, 1936) the probasidium is a saccate structure produced laterally on a hypha. Following karyogamy, during which nucleolar fusion occurs, the diploid nucleus enlarges considerably, showing a single

distinct nucleolus and chromatin threads. It then contracts into a denser structure prior to migrating into the developing basidium. The nucleus again increases in size within the basidium. A spindle with polar centrosomes appears as the nucleolus degenerates. The haploid number of chromosomes in *H. lagerheimi* is probably 5. A typical transversely septate four-celled basidium develops after the second meiotic division is completed. In a study of the nuclear cycle in *Herpobasidium filicinum*, Jackson (1935) observed that the mycelial cells, haustoria and young basidia of this leaf parasite are binucleate. Karyogamy is followed by a single nuclear division in the basidium, after which the latter becomes divided by a single septum into two uninucleate cells. Two uninucleate basidiospores are produced. It is suggested that the second meiotic division may occur at germination of the basidiospore and that the latter probably gives rise to a dikaryotic mycelium. The similarity between this development and that in *Septobasidium sinuosum* is striking.

The globose basidia of the Tremellaceae are commonly divided by vertical septa into four uninucleate cells. The septa appear typically to form at right angles to the long axis of the spindles, although Whelden has reported that nuclear divisions and septum formation are frequently not coordinated. Whelden (1934, 1935*a*, 1935*b*, 1935*c*, 1937) undertook a series of cytological studies on various members of this family. In four species of *Tremella* (1934, 1935*c*), four of *Exidia* (1935*a*) and in *Protodontia uda* and *Tremellodendron candidum* (1937) he reported 4 chromosomes as the haploid number, whereas in three species of *Sebacina* (1935*b*) he found 3. However, Whelden's interpretation of the meiotic figures is difficult to comprehend. The unnecessary reference to the early prophase chromosomes of the first meiotic division as " prochromosomes " is particularly confusing. Furthermore, the latter were reported to condense into short stubby chromosomes, 6 in *Sebacina* and 8 in the other genera, which then become paired. It is possible that the " prochromosomes " are presynapsis univalents and that synapsis begins when the chromosomes are much-condensed, as in *Neurospora* (McClintock, 1945), or they may be heterochromatic bodies. However, a more detailed study is needed before a final conclusion can be reached. Wittlake (1938) observed that there are 8 pairs of chromosomes during meiotic prophase in *Sebacina*

calcea and that in both divisions in the basidium 8 chromosomes pass to each spindle pole. Centrosomes are reportedly present at the spindle poles. Hagerup (1944), who described karyogamy and meiosis in *Tremellodon gelatinosum*, found 4 bivalents at meiotic prophase and 4 chromosomes migrating to each pole at anaphase.

The Tulasnellaceae are an unusual group of resupinate jelly fungi. The basidia, instead of giving rise directly to typical sterigmata, produce sporoid structures, usually four in number, each of which in turn produces a basidiospore. These sporoid structures have been variously referred to by Rogers as "basidial appendages" and "epibasidia", whereas Linder (1940) considered them to be inflated sterigmata. Four species of *Tulasnella* and one of *Gloeotulasnella* were studied cytologically by Rogers (1932). In all species binucleate hyphal cells give rise to binucleate probasidia in which karyogamy occurs. Two meiotic divisions, with transverse spindles, take place in the basidium, following which the four nuclei pass singly into the four basidial appendages. Each appendage becomes separated from the main part of the basidium by a cross wall at its narrowed base. In the four species of *Tulasnella* there is a third nuclear division in each basidial appendage, and the basidiospore which develops from it is binucleate, but in *Gloeotulasnella pinicola* (*T. tulasnei*) no such division was observed.

The Dacrymycetaceae are chracterized by the production of elongate two-sterigmate basidia without septa. Their true phylogenetic relationships with other groups of jelly fungi are obscure, and some investigators have accordingly considered it advisable to place them in a separate order. Only a few cytological studies of these fungi have been made. Bodman (1938) has studied the cytological development of *Dacryopinax* (*Guepinia*) *spathularia*, in which the young binucleate basidia arise from hyphae with binucleate cells on which clamp connections are lacking. Karyogamy is followed by two meiotic divisions, during which the spindles are vertically oriented in the narrow basidium. The chromosome number was not determined, although the haploid number was greater than 2. Wager had earlier reported 4 chromosomes in a species of *Dacrymyces*. The nuclear behavior which follows is rather unusual. The basidium produces two large sterigmata ("epibasidia"), into each of which two of the four nuclei migrate.

Following this, a single nucleus migrates into the developing basidiospore, while the other degenerates in the sterigma. After the spore is discharged, a mitotic division occurs and the spore becomes divided into two uninucleate cells.

There have been very few studies on the subject of homothallism and heterothallism in the Tremellales. Kniep, in his report on *Sebacina calcea* in 1922, was probably the first to demonstrate heterothallism in a jelly fungus. Barnett (1937) was able to grow several species of *Exidia* and one of *Auricularia* in culture. Fertile fruiting bodies were obtained from dikaryotic mycelia in *E. nucleata* and *A. auricula*. In *E. saccharina* the monokaryotic mycelium produces uninucleate oidia, while the dikaryotic mycelium produces binucleate ones. Results obtained from pairing monosporous cultures showed that all species exhibited a bipolar type of heterothallism, and in several cases evidence indicating the existence of multiple allelomorphs at the compatibility locus was obtained.

Vandendries (1937*a*, 1937*b*) considered the basidiospores of *Calocera cornea* to be dikaryotic. A cross wall develops and divides the binucleate spore into two uninucleate cells. Each cell reportedly gives rise to a homokaryotic mycelium with uninucleate cells. Later, anastomoses between the two mycelia produce the dikaryotic mycelium. Conidia produced by homokaryotic mycelia are uninucleate. Single-conidial cultures do not give rise to dikaryotic mycelia, but mixed conidial cultures do. The results obtained by Chu (1949) are not in agreement with those of Vandendries. Chu studied several species of Dacrymycetaceae, including *C. cornea*, and reported that the basidiospores in all species are uninucleate at origin and later become divided into two or more cells after mitosis. By pairing compatible single-spore cultures, Chu found that *C. cornea*, *Dacrymyces deliquescens* and *Femsjonia luteoalba* are heterothallic. A tetrapolar type of heterothallism was discovered in *C. cornea* and possibly in *F. luteoalba*.

It was noted by Olive (1952) that the Sporobolomycetaceae show certain relationships with the Tremellales and should be placed in the heterobasidiomycetes. Martin (1952) has included them in the Tremellales. Actually there is not enough known about the group to ascertain exactly where it belongs. These fungi are characterized by the production of ballistospores which readily

germinate by repetition. The spores resemble basidiospores, but since they are not produced by true basidia they can not be termed such. A pseudomycelium or a true mycelium may also be produced. Guilliermond (1927*a*) reported that the vegetative cells of *Sporobolomyces* are uninucleate and that there is no evidence of a dikaryotic condition or of nuclear fusion in the life cycle. Until recently it has been generally believed that karyogamy does not occur in the life cycle of sporobolomycetes. However, Nyland (1948, 1949) has expressed the opinion that karyogamy occurs in *Sporidiobolus johnsonii* and *Itersonilia pyriformis*, although he did not actually observe the process. Sainclivier (1951*a*, 1951*b*) has claimed that plasmogamy, karyogamy and meiosis occur in *Sporobolomyces*, but the evidence, including a confusing array of drawings and photographs, is far from convincing. It is not clear whether Sainclivier considers the haploid chromosome number to be 2 or 4. The cytological development of *I. perplexans*, a species very similar to *I. pyriformis*, was studied by Olive (1952) who found that the species exists primarily in the dikaryophase. The mycelium is composed of binucleate cells with typical clamp connections. Binucleate sporogenous cells arising from this mycelium produce binucleate ballistospores, each of which may reproduce the dikaryotic mycelium. Binucleate chlamydospores are also produced. Under certain conditions the mycelium gives rise to uninucleate conidia which may be maintained as yeast-like budding cells on certain media or as mycelia on other media. Mycelia from single conidia are homokaryotic and composed of uninucleate cells with incomplete clamp connections. Various combinations of single-conidial mycelia were tested, but these for some undetermined reason never gave rise to dikaryotic mycelia. At no stage in the life cycle was either karyogamy or meiosis observed. Recent studies have indicated that two distinct varieties or species may have been involved in this cytological investigation.

HYMENOMYCETES. The great majority of these fungi are heterothallic, and a typical life history (Figure 60) involves the production of monokaryon (primary) mycelia by the germinating basidiospores. These mycelia have uninucleate cells and lack clamp connections. When two compatible mycelia are paired, anastomoses occur, followed by a pairing of unlike nuclei, and, while this paired condition is maintained by conjugate nuclear division, the dikar-

yotic (secondary) mycelium with binucleate cells and clamp connections is produced. Oidia may also assist in dikaryotization. It is the dikaryotic mycelium which gives rise to the fruiting bodies. Karyogamy and meiosis occur in the basidia, and the basidiospores, usually four in number, are the first cells of the monokaryophase. Initiation of the dikaryophase in *Coprinus lagopus* was studied in considerable detail by Buller (1930, 1931) who believed that a single nucleus from one mycelium could dikaryotize (" diploidize ") a compatible monokaryon mycelium by repeated divisions and nuclear migrations. He found that the dikaryophase could be initiated in a monokaryon mycelium, not only by a compatible monokaryon mycelium, but also by a dikaryon mycelium. Buller also found that nuclear migration during dikaryotization generally proceeds at the rate of about 0.9 mm. per hour in *C. lagopus* and that the maximum rate is about 1.5 mm. per hour. The appearance of clamp connections was used as the criterion indicating that dikaryotization had occurred. This criterion also enabled Buller to obtain some information on the rate of conjugate nuclear division in a dikaryotic hypha. He found the process to occur at the rate of one conjugate division every 50 minutes. Many of Buller's observations were confirmed by Noble (1937) in her studies of *Typhula trifolii.* Here the transverse septa in the hyphae were reported as wholly or partially dissolving during nuclear migration. In an anastomosis between dikaryotic and monokaryotic mycelia, she believed that the dikaryon from the former displaced the monokaryon of the latter.

Quintanilha (1932, 1941) advanced the theory that in certain incompatible mycelial matings in *Coprinus,* involving dikaryotic and monokaryotic mycelia, the subsequent initiation of the dikaryophase in the monokaryotic mycelium could be accounted for by a preceding chromosomal interchange between two nuclei during conjugate division. This unusual idea has never received genetic verification, and cytological evidence is completely lacking. A rebuttal to Quintanilha's claim has been published by Chow (1934). A number of investigators have reported obtaining dikaryotic mycelia by pairing monokaryon mycelia which are not completely compatible; that is, the two compatibility allelomorphs at one locus are alike, while at the other locus they are unlike. The normal dikaryotic mycelium of *Pleurotus cornucopioides* has binucleate cells and

true clamps, but when two partially compatible monokaryon mycelia are paired, a mycelium with false clamp connections develops. The dikaryon is maintained only in the terminal hyphal cell, where, at each conjugate division, one nucleus is retained in the incomplete clamp and the cells of the mycelium remain uninucleate (Vandendries, 1937*b*).

The dikaryotic mycelium of the heterothallic *Psilocybe coprophila* produces oidia, some of which are dikaryotic and others of which are homokaryotic (Gilmore, 1926). A most interesting experiment with some bearing upon cytoplasmic inheritance was carried out by Harder (1927) upon the dikaryotic mycelia of *Schizophyllum commune* and *Pholiota mutabilis*. By micromanipulation at the proper stage of development, Harder was able to obtain, from a single clamp or from the adjacent penultimate cell, a mycelium which contained only one of the two types of nuclei of the dikaryon, but which presumably contained cytoplasm of both the parent mycelia. Some of these mycelia did not resemble either of the two distinct monokaryon mycelia from which the dikaryon mycelium was derived, but showed intergradations between the two, while some resembled more the strain whose nuclei were no longer present in their hyphae. Although Harder's work has received little attention, it does indicate that the higher basidiomycetes may prove to be interesting subjects for comparative studies in cytoplasmic and nuclear influences.

Not all hymenomycetes develop in the typical manner described earlier in this section. According to Nisikado (1930), the hyphal cells of *Stereum hirsutum*, a species which Kniep earlier found to be homothallic, are multinucleate, and clamp connections occur singly or in whorls of two to four at the septa. A pair of conjugate nuclei was found to be associated with the formation of each clamp, and as many as three or four pairs were observed in conjugate division in the same area. Single nuclear divisions also occur independent of clamp formation. A mycelium similar in appearance has been described in the homothallic *Coniophora puteana* (*C. cerebella*) (Kemper, 1937; Greis, 1937*c*). However, in this species the nuclei are not distinctly paired, and there is no true conjugate nuclear division. Formation of the clamps, which occur one to four at a septum, is not coordinated with the nuclear divisions.

According to Fukano (1932), the vegetative hyphae of *Hypoch-*

nus sasakii, supposedly a homothallic species, are without clamps and the cells are multinucleate. The hyphal cells giving rise to the basidia, however, are binucleate, and basidial development is typical. As a vegetative hypha grows, all the nuclei in the tip cell generally come together at a common point in the cell and divide conjugately, following which a cross wall divides the tip into two cells with equal numbers of nuclei in each. In a cytological study of *Sclerotium rolfsii*, which was suspected of being heterothallic, Goto (1936) showed that the secondary mycelium is composed of multinucleate cells with clamp connections that are frequently multinucleate. Nuclear divisions in these hyphae appear to occur primarily in the regions of the developing clamps which usually appear singly at the septa. It is interesting to note that hyphae submerged in the agar medium or those within the host tissues are devoid of clamp connections, as are the binucleate subhymenial cells.

After an extensive study of *Agaricus campestris* f. *bisporus*, Kligman (1943) found that the cultivated agaric, like the wild four-spored form (Klioushnikova, 1939), is probably homothallic. No clamp connections and no conjugate nuclei were observed in the mycelium, the cells of which are multinucleate. A rather high degree (20–30%) of sterility was found among single-spore cultures. When these sterile cultures were paired in various combinations, they generally remained sterile, but a few such combinations gave rise to fruiting bodies. Kligman was unable to prove by genetic analysis that heterokaryosis and heterozygosity had taken place. Although the majority of evidence strongly indicates that the cultivated mushroom is homothallic, certain of Kligman's findings suggest that heterokaryosis and hybridization may be possible with cultures differing in morphological and physiological characters. Kühner (1950) studied a number of species of *Phellinus* (*Fomes*), none of which produces clamp connections, and observed that in some species the mycelium is composed of binucleate cells, while in others the cells are multinucleate. It was found that nuclear divisions might be synchronous only when the number of nuclei is five or less.

In a summary of the various types of development in the basidiomycetes, Vandendries (1937*a*) included the following: (*a*) parthenogenesis (haplo-parthenogenesis), (*b*) homothallism without

clamp connections, (*c*) homothallism with clamps (but representative species is miktohaplontic, not truly homothallic), (*d*) heterothallism without clamps, (*e*) heterothallism (bipolar and tetrapolar) with clamps. Some investigators have concluded that certain agarics are homothallic in view of the fact that single-spore cultures produce clamp connections. Such a conclusion is hazardous unless further studies support it. It is possible for the clamps to be incomplete and for the hyphal cells to be uninucleate. Also, there may be more than one type of development in the same species if we accept the findings of Mounce (1921, 1922) who has reported both homothallism and heterothallism in *Coprinus lagopus* and *C. niveus*, and of Biggs (1938) who reported similar discoveries in *Peniophora ludoviciana*[2]. It should also be noted that the production of fertile fruiting bodies by a single-spore culture does not necessarily demonstrate true homothallism, since karyogamy and meiosis may fail to occur in the life cycle, as will be described in greater detail presently.

Although most of the recent cytological studies of basidial development in the hymenomycetes have been confined mainly to the Agaricaceae, a number of studies deserving of mention have been made upon species belonging to other groups. Kharbush (1929) studied the cytological developments in three species of *Exobasidium*, with particular attention given to *E. discoideum*, and observed that the parasitic thallus is composed of binucleate cells without clamp connections. Karyogamy occurs in the young basidium and is followed by meiotic prophase. A centrosome reportedly divides, and each half takes its position at a pole of the developing spindle. The chromatin during late prophase was described as taking the form of numerous granules—interpreted as "protochromosomes"—which eventually come together and form 2 large chromosomes. Two chromosomes were thought to pass to each pole of the vertical spindles in both the first and second divisions. The four haploid nuclei pass into four basidiospores. The evidence for the existence of "protochromosomes" is no more convincing in *Exobasidium* than in other fungi in which they have

[2] Lange (Dansk. Bot. Ark. 14: 1–164. 1952) has proposed the term "amphithallism" to designate species in which both heterothallic and homothallic mycelia may be obtained from the same fruiting body. He found the concept applicable to three species of *Coprinus*.

been reported. It appears more likely that these are the true chromosomes or that they are heterochromatic bodies on the chromosomes. The haploid chromosome number is probably greater than 2.

Bose (1937) observed a great deal of consistency in the cytology of basidial development in 11 species of polypores studied by him. In all of them karyogamy is followed by two meiotic divsions, and four uninucleate basidiospores are produced. His report (1935) of amitotic fragmentation of some of the fusion nuclei in *Ganoderma* must be held in question until further investigations have been performed.

Following karyogamy in *Corticium rolfsii*, Goto (1936) observed characteristic stages in meiotic prophase. Four bivalents are apparent at diakinesis and on the metaphase plate, and 4 chromosomes pass to each spindle pole. Centrosome-like bodies are also present. When the two meiotic divisions have been completed, a third division occurs in the basidium or in the basidiospores. In his study of a species of *Peniophora*, Whelden (1936) reported that 8 chromosomes are occasionally "vaguely associated in pairs" during meiotic prophase. Since he did not actually observe nuclear spindles, it is difficult to interpret some of the stages illustrated by him. It is not clear whether the haploid chromosome number is 4 or 8. Judging from the position of the nuclei immediately following the first division in the basidium, one might conclude that the spindle is transverse. It is interesting to note that the cytology of cystidial development is very similar to that of basidial development up to the end of the second nuclear division. Kemper's study (1937) of meiosis in *Coniophora puteana* and *C. arida* led him to conclude that the haploid chromosome number is 4. The four uninucleate basidiospores produced by each basidium become binucleate following mitotic nuclear division. In *Peniophora ludoviciana* Biggs (1938) has reported heterothallism of the bipolar type, but she also found that fertile sporophores are produced by monokaryon mycelia from single basidiospores. Her cytological studies of the homothallic type of development demonstrated that karyogamy (involving homodikaryons) and meiosis occur in the basidium. A third mitotic division in the basidium produces eight nuclei, but only four nuclei pass into the four basidiospores, while the other four nuclei degenerate in the basidium. Biggs considered

the condition in *P. ludoviciana* as evidence that homothallic forms have arisen from heterothallic forms through suppression of a self-sterility mechanism.

Aleurodiscus canadensis (Skolko, 1944) produces mostly two-spored basidia in addition to a smaller number of one-spored and three-spored ones. Cytological studies have shown that karyogamy is followed by two meiotic divisions, with spindles oblique to transverse, and four nuclei are produced in the basidium. When the basidium forms two spores, two nuclei pass into each spore. These spores produce mycelia with clamp connections. In the three-spored basidium, two spores are apparently uninucleate at their origin, while one is binucleate. The first type of spore produces a mycelium without clamps, but two compatible mycelia of this type will, when paired, give rise to a typical dikaryophase. This fungus, like a number of others already discussed, typically exists vegetatively as a miktohaplont.

A peculiar behavior of the post-reductional nuclei has been reported in the basidium of *Sistotrema confluens* by Kühner (1947). This species commonly produces basidia with five to eight uninucleate spores. The two characteristic meiotic divisions occur in the basidium, but there is a third division reportedly in the sterigma or in the basidiospore, after which one nucleus from each mitotic division migrates back into the basidium. If less than eight spores are formed, a complementary number of supernumerary nuclei remains in the basidium.

Baker (1941) has investigated the nuclear cytology of *Physalacria inflata* and reported that the hyphal cells and most of the elements of the fructification are binucleate. Karyogamy is followed by two meiotic divisions, and the basidium produces four uninucleate spores. Nuclear fusion, followed by a single nuclear division, was also observed in the cystidia. In *P. andina* the basidiospores are binucleate and may be miktohaplontic, since they arise from two-sterigmate basidia.

A large number of species of the Agaricaceae and other homobasidiomycetes, including several gasteromycetes, were examined cytologically by Wakayama (1930*a*, 1932) whose untimely death ended his important contributions to the cytology of the fungi. Nuclear phenomena in the basidia of all species studied by him were essentially uniform. Only a single chromatic body, probably

representing the nucleolus with the chromatin collapsed around it, was seen in each of the two haploid nuclei at the beginning of karyogamy. Nucleolar fusion is followed by the appearance of the chromatin in a " reticulate " arrangement. Eventually the chromatin appears in the form of distinct and elongate leptotene chromosomes which pair. Thus, synapsis, as reported here, occurs later than it was observed by McClintock (1945) and Singleton (1953) in *Neurospora* or by Olive (1949*a*) in *Coleosporium*. Wakayama's illustrations of the early prophase chromosomes frequently indicate that they may be distinctly heteropycnotic. The paired chromosomes are often considerably twisted about each other, and at diakinesis well-defined condensed bivalents are visible in addition to the large nucleolus. An intranuclear spindle with polar centrosomes appears, and the nuclear membrane breaks down. Wakayama, like a number of other investigators, considered the presence of centrosomes as indicative of primitiveness in the fungi, since these structures are found in animals, but generally not in higher plants. The bivalents become arranged at the equator of the spindle at metaphase and separate somewhat irregularly to opposite poles at anaphase, while the nucleolus degenerates. In most cases the second meiotic division proceeds without a distinct interkinetic phase. Each basidiospore receives a single nucleus which in several species divides mitotically within the spore. In a few cases the third division takes place in the basidium. Of the 25 species of agarics studied by Wakayama, eight were reported with a haploid chromosome count of 6, 14 with 4, and three with 2. Six of the seven species of Polyporaceae reportedly have 4 and one has 6. All six species of *Clavaria* were found to have 4, and the single species of the Thelephoraceae investigated (*Craterellus cornucopioides*) has 4. Wakayama (1930*a*) has also listed the chromosome numbers previously reported in the Agaricaceae. One of the more interesting observations made by this investigator concerns the possibility of polyploidy. Since *Hypholoma fasciculare* was found to have 2 chromosomes, while the closely related *H. appendiculatum* has 6, the latter was considered a triploid species. Likewise *Tricholoma melaleucum*, with 4 chromosomes, may be a diploid species, since *T. rutilans* has 2 chromosomes.

The cytological details of basidial development described by most other investigators for other species of agarics are, with a few

exceptions, in general agreement with the scheme presented by Wakayama. Apparently no one has convincingly demonstrated at exactly what point synapsis begins, although the report of Ehrlich and McDonough (1949) on *Schizophyllum commune* indicates that it may occur earlier than Wakayama reported it. This lack of concise information on synapsis is a reflection on the small size of the prefusion nuclei. The fusion nucleus enlarges considerably after karyogamy, and later stages in development may be observed with greater facility. In general the spindles in the basidium appear to have polar centrosomes, and their orientation is transverse to oblique. Haploid chromosome numbers reported for various agarics by recent investigators other than Wakayama are listed as follows: *Agaricus campestris*, 4 (Sass, 1928; Sarazin, 1938), 9 (Colson, 1935); *Coprinus ephemerus* f. *bisporus*, 8–10 (Sass, 1929); *C. atramentarius*, 4 (Vokes, 1931), *C. hendersonii*, *C. lagopus* and *C. tomentosus*, 2 ? (Chow, 1934); *Camarophyllus virgineus*, 4 (Bauch, 1926); *Lentinus variabilis*, 2 or more (Kühner, 1928), *Lepiota acutesquamosa*, 6 (Greis, 1937*a*), *L. lenticularis*, 4 (Hagerup, 1945); *Lactarius vellereus*, 4 (Salmon, 1937); *Russula emetica*, 4 (Ritchie, 1941); *Amanita caesarea*, 4 (Ritchie, 1948*b*); *Schizophyllum commune*, 3 (Ehrlich and McDonough, 1949). Chow's report of 2 chromosomes in *Coprinus* is questioned, since several of the illustrations of meiotic prophase indicate that the number is more likely 4. It should be noted that reports of odd numbers of chromosomes in the basidiomycetes are rare, as are reports of haploid numbers greater than 8. The number most frequently found is probably 4. Colson's report of 9 for *Agaricus campestris* and Sass's report of 8 to 10 for *Coprinus ephemerus* f. *bisporus* apparently represent the largest numbers thus far discovered in the autobasidiomycetes.

A number of species of agarics are known to have two-spored forms, often classed as varieties, in addition to the typical four-spored forms. The cytology of several of these has been described. The best known representative of the two-spored forms is the commercially grown mushroom *Agaricus campestris* f. *bisporus*. As has already been pointed out (Kligman, 1943), the mycelial cells are multinucleate with no conclusive evidence of conjugate nuclear pairs. Sass (1928), who studied the cytology of basidial development in this fungus, found that karyogamy occurs and is followed

by the usual two nuclear divisions. A pair of nuclei passes into each spore. Sass, like Kligman, found no evidence of conjugate nuclear pairs in the mycelium developing from these spores. However, in *Coprinus ephemerus* f. *bisporus* he (1929) obtained evidence which indicates that the two basidiospores contain dikaryons. The hypha arising from the germinating spore is at first coenocytic, but it soon develops into a mycelium with uninucleate cells. The dikaryotic mycelium with clamp connections arises from this, probably by means of hyphal anastomosis. A similar condition was described for two-spored forms of *Naucoria semiorbicularis* and *Galera tenera.* In all three species the basidial cytology is typical and rather uniform in the four-spored type as well as in the two-spored type up to the time of spore formation. The four-spored types are distinctly heterothallic, and it is obvious that the two-spored forms have dikaryotic basidiospores and are miktohaplontic.

Both Smith (1934) and Kühner (1938) have reported, in a number of two-spored species of *Mycena,* a type of cytological development similar to that described above. They have also described typical basidial development in the four-spored forms. In *M. murina,* however, Smith found that, following a third nuclear division in the basidium, each of the four spores receives two nuclei. It was not demonstrated whether these nuclear pairs are homo- or heterodikaryons. Still another type of development, recognized as haplo-parthenogenesis by Kühner, was described by both investigators for a considerable number of species of *Mycena* and by Bauch (1926) in a two-spored form of *Camarophyllus virgineus.* The mycelial cells and the basidia are uninucleate and karyogamy fails to occur. In most cases two successive mitotic divisions take place in the basidium. In most of these species, according to Smith, two binucleate spores are produced, but basidia with up to four spores (uninucleate) were found. Kühner observed that a number of such species commonly produce four uninucleate spores, whereas in a few other species there is only a single nuclear division in the basidium, and two uninucleate spores are produced. Obviously, there is no sexuality in this type of life cycle. Such development is found in all the main groups of fungi and is frequently referred to as "parthenogenesis". The term "haplo-parthenogenesis" is more concise in view of the fact that there is no doubling of the chromosome number in the life cycle.

A number of investigators have described in the basidium certain bodies other than the nuclei which are often associated with the latter. Some of these structures reportedly assist in spore formation. Vokes (1931) found that each of the four haploid nuclei in the basidium of *Coprinus atramentarius* is attached by a thin strand to a hyaline body which in turn is attached to the wall of the basidium. Each of the hyaline bodies was believed to act upon the wall in such a manner that the latter is pushed up into a sterigma. The nucleus then follows the hyaline body into the basidiospore. Colson (1935) reported that there are no such bodies or connecting strands in *Agaricus campestris*, and Ritchie (1941), who used a large number of fixatives in his study of *Russula emetica*, came to the conclusion that the darkly staining strands which he sometimes observed extending from the basidial nuclei to the basidial apex were probably artifacts resulting from certain types of fixation. Some investigators have described the particpation of centrosomes in basidospore formation, but Sass (1935) has questioned these reports. In a cytological study of sterile fructifications of *Coprinus sterquilinus*, he found that most of the basidia contain only two nuclei but produce four sterigmata and four enucleate basidiospores. Since only one nuclear division occurs in each of these basidia and the centrosomes disappear thereafter, Sass has expressed doubt that centrosomes function in spore formation.

Sass (1934) found evidence of a Golgi apparatus in *Coprinus sterquilinus*. It was described as consisting of a large hyaline body, or " Nebenkern ", containing one or more chromatic elements in the form of " Golgi crescents " or granules. Prior to the division of the fusion nucleus, which attains a diameter of 10 μ, this body divides into four similar but smaller bodies. The latter are at first located near the base of the basidium but later migrate to the apex where they become associated with the developing sterigmata and spores. They lose their chromaticity as the spores develop, and apparently the Golgi crescents become incorporated into the spores. Ritchie (1948) has described similar bodies in *Amanita caesarea*.

Sass's report (1929) on basidospore germination in *Coprinus ephemerus* f. *bisporus* is deserving of especial attention, since this type of development is probably of wider occurrence and of greater importance than is commonly recognized. It has already been

pointed out that, although the basidiospore is obviously dikaryotic, containing a heterodikaryon in this case, it gives rise to a mycelium which is at first composed of uninucleate cells without clamp connections and from which the dikaryophase with its clamp connections arises, probably by means of anastomoses between hyphal cells that contain nuclei of compatible types. It would appear that such a fungus has a certain advantage over the usual miktohaplont, since this separation of the two types of nuclei into different hyphae of the same mycelium would enhance the opportunity for outbreeding. Yet, if other compatible mycelia are not available, the fungus is assured of a means of completing its life cycle. The same situation appears to exist in the two-spored form of *Galera tenera* (Vandendries, 1937*a*), and a fairly similar condition has been described in certain smut fungi. A variation of this type of development is found in *Pholiota aurivella* (Martens, 1932; Martens and Vandendries, 1933). In this agaric the dikaryotic mycelium produces a type of conidium which contains a dikaryon. Each conidium becomes divided into two uninucleate cells which may break apart. Upon germination, if the cells remain together, the cross wall between them is dissolved and the same dikaryon is restored. If the cells have separated, each gives rise to a monokaryon mycelium which then has the opportunity to anastomose with any available compatible mycelium to produce the dikaryophase. This also is a mechanism which permits outbreeding. The species is characterized by a bipolar type of heterothallism.

It is rather surprising that there have been so few genetic investigations of the hymenomycetes other than those concerned with the compatibility factors and their allelomorphs. Jordanoff (1932) treated *Coprinus* with narcotic substances and was able to induce certain changes in development by applying treatment at the time of meiosis. Treatment between the first and second divisions resulted in the production of some basidia with more than four spores, a third division being induced to occur in the basidium. Treatment immediately after the second nuclear division led to the production of some basidia with less than four spores, some of which proved to be heterokaryotic for the compatibility factors. Apparently, none of these changes was the result of mutation, since all cultures from these basidiospores reverted to normal in the next generation. Sass (1932) found a sterile *Coprinus*, resembling *C.*

boudieri, whose sporophores failed to form basidospores and did not undergo deliquescence. The mycelium produced clamp connections. A cytological study demonstrated karyogamy in the young basidium, but the basidium soon collapsed and meiosis failed to occur. Failure of the basidium to develop further has some resemblance to ascus abortion in certain strains of *Neurospora*, as described by Dodge. However, ascus abortion in *Neurospora* does not occur until after the fusion nucleus has undergone its usual successive meiotic and mitotic divisions. In this species of *Coprinus* basidial abortion appears attributable to a truly diploid condition. If the fungus should prove to be heterothallic, as it probably is, hybridization studies employing normal and mutant strains should be of exceptional interest.

GASTEROMYCETES. This group of homobasidiomycetes is distinguished from the foregoing in having the hymenium enclosed within a peridium and in lacking the spore discharge mechanism. There is a great deal of conformity in the nuclear developments in the two groups. In general, the same mycelial phases, monokaryotic and dikaryotic, exist here as in the hymenomycetes. The available evidence indicates that the majority of species are heterothallic. Probably tetrapolar heterothallism with multiple allelomorphs is most prevalent. The cytology of basidial development is essentially like that characteristic of the hymenomycetes, as Wakayama (1932) has emphasized. Karyogamy in the young basidium is followed generally by two meiotic divisions, and four haploid nuclei are produced. Frequently a third division, mitotic in nature, occurs in the basidiospore before it becomes disconnected from the basidium. Occasionally, as in *Sphaerobolus tubulosus* (Lorenz, 1933), there is a third division in the basidium, after which four of the nuclei pass into four basidiospores and the other four nuclei degenerate in the basidium. In *Lysurus sulcatus* (White, 1944) a four-nucleate basidium produces four spores which remain uninucleate.

The meiotic spindles are almost invariably transversely oriented in the basidium. A number of investigators have reported centrosomes at the spindle poles. In *Calvatia candida* (Brandza and Solacolu, 1937) a centrosome was observed in the nucleus during late prophase, where it divides at the time of spindle formation. Lander (1935) noted that, following the second meiotic division in

the basidium of *Pisolithus tinctorius*, centrosome-like bodies with fibers attaching them to their respective nuclei may be seen in contact with the basidial wall where the sterigmata are forming. These bodies were found to precede the nuclei into the spores. A somewhat similar situation was described by Lander (1933) in *Scleroderma lycoperdoides*. The phenomenon resembles that described earlier by Vokes (1931) in *Coprinus atramentarius*.

The length of the sterigmata in the gasteromycetes is quite variable. In some species the spores are frequently sessile, while in others they are produced on short or long sterigmata, which often vary in length in the same species. In species that produce long narrow sterigmata one may observe the tendency of the nuclei to become greatly attenuated as they pass into the basidiospores. The most extreme case of nuclear attenuation reported in the fungi, as far as the writer is aware, is that described by Lander (1934) in *Arachnion album*, where the nuclei in the long sterigmata appear as finely drawn out filaments. A somewhat similar condition was described by Ritchie (1948*a*) in *Lycoperdon oblongisporum*.

Since even the diploid nuclei of the gasteromycetes are often comparatively small, there have been relatively few detailed studies of nuclear divisions in the group. However, several investigators have attempted determinations of chromosome numbers. The haploid chromosome numbers, as reported by these authors for various species of gasteromycetes, are listed as follows: *Lycoperdon gemmatum*, 2 (Wakayama, 1932); *Lasiosphaera fenzlii*, 4 (Wakayama, 1932); *Geastrum hygrometricum*, 2 (Wakayama, 1932), *G. fimbriatum*, 2 (Brandza and Solacolu, 1937); *Sphaerobolus tubulosus*, 3 or 4 (Lorenz, 1933); *Scleroderma lycoperdoides*, 2 (Lander, 1933); *Pisolithus tinctorius*, 4 (Lander, 1935); *Calvatia candida*, 2 (Brandza and Solacolu, 1937); *Tylostoma mammosum*, 4 (Greis, 1937*b*).

CONCLUSIONS

1. The nucleus in all major groups of fungi is essentially the same in structure and is similar in structure to the nucleus of higher organisms. The nucleus typically contains a single nucleolus, chromatin strands, karyolymph and a nuclear membrane.

2. Mitotic divisions occurring in the vegetative phase of the fungi are essentially the same as in higher organisms, although the

details are frequently obscured by the small size of the nuclei. The spindles appear to be intranuclear in origin. In the Plasmodiophorales there is an unusual type of mitosis known as "promitosis", during which a ring of chromosomes separates to each pole as the nucleolus divides.

3. Meiotic nuclear divisions have been the best source of information concerning the structure and behavior of fungus nuclei. Meiosis is generally directly preceded by karyogamy. Karyogamy, stages in meiotic prophase and the two meiotic divisions proceed very much as in higher organisms. The spindles, which are intranuclear, are frequently described as being provided with polar centrosomes. Most haploid chromosome counts fall within the range of 2 to 28, with numbers less than 10 predominating. Heteroploidy has been convincingly demonstrated in only a few species of fungi.

4. In most phycomycetes, the haplophase, consisting of coenocytic hyphae with many small haploid nuclei, appears to predominate, with the diplophase being confined to the zygote, in which karyogamy occurs. Meiosis is generally believed to occur during germination of the zygote. However, there is need of more convincing cytological and genetic evidence in support of this idea for most groups of phycomycetes.

5. In the majority of ascomycetes and basidiomycetes three phases of nuclear associations exist: haplophase, dikaryophase, and diplophase. In the dikaryophase the nuclei are associated in conjugate pairs, the diplophase being confined to the zygote (young ascus or probasidium). In both groups meiosis has been demonstrated, both cytologically and genetically, to occur in the ascus and basidium, respectively.

6. Notable exceptions to the characteristic life cycles outlined above are found in *Allomyces* which has an alternation of well-developed gametophytic (haploid) and sporophytic (diploid) generations, and in certain yeasts whose vegetative phase is predominantly diploid.

7. It appears quite probable that heterokaryosis and hybridization are of common occurrence, not only in heterothallic fungi, but also in many homothallic species.

8. It is very doubtful that amitosis occurs as a normal process in any of the fungi.

9. The chromocenters of the vegetative nuclei are probably homologous with heterochromatic bodies observed on the chromosomes at meiotic prophase and with the so-called "protochromosomes" of certain authors.

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ADDENDUM

The Acrasiales, which are considered by some authorities to be related to the myxomycetes, have been the subject of considerable debate regarding the presence or absence of sexuality in the life cycle. In 1920, Skupienski claimed that sexual cell fusions take place in *Dictyostelium mucoroides*, but his conclusions were not widely accepted by most later investigators. However, Wilson (1952), with the aid of the orcein technique, has confirmed the occurrence of sexual reproduction in the group. He has found that plasmogamy occurs in *D. discoideum* between pairs of amoeboid gametes at the aggregation stage prior to sorocarp formation. Nuclear fusions occur shortly thereafter, followed by meiosis. But the four-nucleate spore-mother-cells thus formed remain undivided until spore formation in the sorocarp. The haploid chromosome number is 7.

Studies on heterokaryon production in the Aspergillaceae have been continued by Pontecorvo et al. (1953). Heterocaryons were obtained in *Aspergillus niger* between mutant cultures differing in several genetic factors. With or without camphor treatment, a small number of conidia were obtained which gave rise to diploid cultures. The fact that these latter cultures were truly diploid is strongly evidenced by the data on somatic segregation and recombination obtained from conidial analysis. These important discoveries demonstrate that it is possible to obtain hybridization and segregation in a fungus lacking the sexual phase. The feasibility of similar experiments in deriving new strains of *Penicillium notatum* and other useful fungi that lack the sexual stage is readily apparent.

Mathieson (1952) has reported an unusual situation in the pyrenomycete, *Chromocrea spinulosa*. The ascus contains four large

and four small two-celled spores, occurring in the six arrangements expected if spore size is controlled by a single pair of allelomorphic factors. Furthermore it was discovered that the larger ascospores give rise to mycelia that are self-fertile and produce asci which again show a similar segregation for spore size. The smaller ascospores produce only self-sterile mycelia. Although perithecial development is stimulated at the line of contact between the two types of cultures, conclusive evidence of hybridization was not obtained. Mathieson's conclusion that "the difference in spore size is a pleiotropic expression of mating type and . . . the fungus is heterothallic" is open to question. The condition here is in some ways comparable to that in the wild type, clumped-perithecial culture of *Glomerella cingulata*, a single-ascospore culture of which produces many asci with spores of clumped-perithecial and scattered-perithecial genotypes. In *Chromocrea*, as in *Glomerella*, this situation appears to be due to a specific gene mutation. In *Chromocrea* the mutation is an obligate one, only a wild-type nucleus and a mutated nucleus being compatible. It is probably incorrect to classify such types of development either as typical homothallism or as true heterothallism. Mathieson observed no sex organs in *C. spinulosa*. He estimated a haploid chromosome number of 8.

In the operculate discomycete, *Humaria leucoloma*, Racovitza and Racovitza (1945) found that the chromosomes become arranged at the equatorial plate at metaphase of the first and second meiotic divisons but pass to the poles irregularly. Centrosomes were observed at the spindle poles. A haploid chromosome count of 6 was made in this species as well as in *H. tetraspora* (Racovitza and Racovitza, 1946). In the latter species the spindles are arranged in conjugate pairs during the second and third divisions in the ascus, much as in *Neurospora tetrasperma*.

In her recent cytological investigations of a large number of ascomycetes, most of them discomycetes, Heim (1951*a*, 1951*b*, 1952) noted that the chromosomes of the vegetative nuclei have conspicuous thickenings called chromocenters. Her assumption that these coincide in number with the chromosomes is open to question, since there is no definite evidence that some chromosomes may not have more than one chromocenter while others may have none. Although no direct correlation is available there is a distinct possibility that the chromocenters of the vegetative nuclei of fungi are homologous with the major heterochromatic regions (possibly

including such structures as the " larger chromomeres " identified in *Neurospora* by Singleton) observed on the chromosomes during meiotic prophase by a number of investigators. It has been shown that in certain species of fungi some chromosomes have no conspicuous heterochromatic regions, whereas others may have one or more. Heim used Heidenhain's haematoxylin, crystal violet, and the Feulgen technique in her staining procedures. Contrary to expectations, the chromocenters were never more than weakly Feulgen-positive. Heim's contention that the " protochromosomes " of certain authors are probably chromocenters appears to be a logical conclusion.

The usual three nuclear divisions in the ascus were found to proceed in typical fashion in all the species studied by Heim and in no species was there any evidence of more than one nuclear fusion in the life cycle. Clearly defined equatorial plate arrangements of chromosomes at metaphase were rarely observed. Centrosomes and astral rays were not seen. The chromosome numbers reported by Heim (1952) for the various ascomycetes that she studied are listed, in accordance with her own taxonomic designations and with the respective binomials recognized by Seaver included in parentheses, as follows: $n = 4$: *Exoascus* (*Taphrina*) *deformans, Melastiza miniata* (*M. charteri*); $n = 6$: *Acetabula vulgaris* (*Paxina acetabulum*), *Anthrocobia melaloma* (*Patella m.*), *Bulgaria inquinans* (*Phaeobulgaria i.*), *Calycella citrina* (*Helotium citrinum*), *Ciliaria* (*Patella*) *hirta, C. scutellata* (*Patella s.*), *Cudonia circinnans, Disciotis venosa* (*Peziza v.*), *Helvella atra, H. crispa, H. lacunosa, Peziza aurantia* (*Aleuria a.*), *Pulvinula constellatio* (*Lamprospora c.*), *P. convexella* (*Lamprospora haemastigma*), *Pustularia ochracea* (*Peziza o.*), *Pyronema confluens* (*P. omphalodes*), *Spathularia flavida* (*S. clavata*); $n = 8$: *Phyllactinia corylea, Sordaria fimicola, Aleuria micropus* (*Peziza m.*), *A. vesiculosa* (*Peziza v.*), *Galactinia saniosa* (*Peziza s.*), *Humaria leucoloma* (*Humarina l.*), *Lachnea hemisphaerica* (*Patella albida*), *Leotia lubrica, Macropodia macropus* (*Paxina hispida*), *Otidea onotica* (*Scodellina leporina*), *Otidea* (*Scodellina*) *vitellina, Sepultaria sumnerii* (*S. arenicola*). As may be noted, the chromosome counts for certain species do not correspond with those obtained by other investigators. Unless it is shown that heteroploidy exists in these fungi, some of these counts must be questioned.

The earlier micrurgical experiments of Harder on dikaryotic

mycelia of certain hymenomycetes have stimulated recent studies in this interesting field. With the use of certain mutant strains and by means of combining compatible mating types in *Collybia velutipes*, Aschan (1952) obtained heterodikaryons for further study. A large number of "neohaplonts" were derived from the uninucleate oidia produced by the dikaryotic mycelia. Although the neohaplont mycelia were all monokaryotic, considerable variation was found to exist among cultures containing nuclei of the same genotype, thus indicating a residual effect of the cytoplasm of the diraryotic mycelium upon the monokaryotic mycelium. Fries and Aschan (1952) synthesized heterodikaryons in *Polyporus abietinus* by combining an old, degenerate strain, d_1, with a compatible, freshly isolated and vigorous strain, b_1. By means of micrurgical operations similar to those performed by Harder and discussed earlier in this paper, they recovered neohaplonts, among which only those carrying the b_1 nuclei survived. The d_1 nuclei were concluded to be incapable of surviving because of unsatisfactory cooperation between them and the mixed cytoplasm of the original dikaryon. It is interesting to note that most of the viable neohaplonts differed markedly in both morphological and physiological characters from either of the original d_1 and b_1 cultures. These studies confirm the earlier findings of Harder.

A review of selected papers on the cytology, genetics, and sexuality of the fungi is contained in a publication by F. Moreau (Les Champignons: physiologie, morphologie, développement et systematique. Encyclopédie Mycologique vol. 22. 940 p. 1953), which appeared too late for review in this paper.

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EXPLANATION OF FIGURES

PLATE I

FIGS. 1–16. *Sorosphaera veronicae*. 1–10. Stages in somatic mitosis; 11–14, first meiotic division; 15 and 16, second meitoic division. × 3000. (After Webb, 1935)

FIGS. 17–25. *Thraustotheca clavata*. 17. Early prophase of mitosis in oogonium, × 2015; 18, late prophase, × 2015; 19, metaphase, × 2015; 20, anaphase, × 2015; 21, oogonium with synchronous mitoses, × 1135; 22, egg delimitation, × 1135; 23, plasmogamy, showing deposition of male nucleus by fertilization tube of antheridium, × 1135; 24, karyogamy, × 1135 and × 2015; 25 zygote (oospore) with fusion nucleus, × 1135. (After Shanor, 1937)

PLATE II

FIG. 26. Life cycle of *Allomyces*, subgenus *Euallomyces*.

FIG. 27. Life cycle of *Allomyces*, subgenus Cystogenes. (After Wilson, 1952)

PLATE III

FIG. 28. *Thraustotheca clavata*, life cycle.

PLATE IV

FIGS. 29–31. Three types of life cycles in the yeasts. 29, *Saccharomyces cerevisiae*; 30, *Saccharomycodes ludwigii*; 31, *Zygosaccharomyces* (?).

FIGS. 32–47. Sexual reproduction in *Glomerella*, involving a cross between two different strains. 32, early stage in perithecial initiation; 33, outer coil beginning to surround inner (ascogonial) coil; 34, appearance of copulation hypha from the second strain; 34, plasmogamy; 36, initiation of crozier development in ascogonial coil; 37–47, crozier and ascus development; 40, meiotic prophase; 41, late anaphase; 42, prophase of second meiotic division; 43, prophase of third nuclear division; 44, anaphase of third division; 45, formation of the eight uninucleate ascospores; 46, mature ascus; 47, perithecium with asci in various stages of development.

PLATE V

FIG. 48. Diagrammatic representation of nuclear behavior during ascus development in 8-spored species of *Neurospora* (with segregation of compatibility factors occurring in first meiotic division). (After Dodge, 1927)

FIG. 49. Diagrammatic representation of nuclear behavior during ascus development in *Neurospora tetrasperma* (with segregation of compatibility factors occurring in the first meiotic division). (After Dodge, 1927)

FIGS. 50 and 50a. Chromosomes of *Neurospora crassa*. 50, mid-pachytene stage of the prophase nucleus, showing major chromomeres of the seven bivalents, × 2400; 50a, prometaphase of the third division in the ascus (s = satellite on chromosome 2), × 2400. (After Singleton, 1953)

FIGS. 51–53. Chromosomes of *Neurospora*. 51, diakinesis in *N. sitophila*, showing seven bivalents, × 3120; 52, metaphase of third nuclear division in the ascus of *N. tetrasperma*, × 6240; 53, mitotic metaphase in ascospore of *Neurospora* sp. (New Zealand stock), × 6240. (After Fincham, 1949, with corrections of magnification supplied by the latter)

FIGS. 54 and 55. Chromosomes of *N. sitophila*. 54, anaphase of first meiotic division, with six univalents at each pole and one lagging bivalent near the center; 55, two metaphase groups of the third nuclear division in the ascus, × 2975. (After Dodge, Singleton, and Rolnick, 1950)

FIGS. 56–58. Stages in first meiotic division in *Aleuria rutilans*. 56, diakinesis, showing 16 bivalents and irregular nucleolus; 57, early anaphase; 58, telophase, × 2285. (After Wilson, 1937)

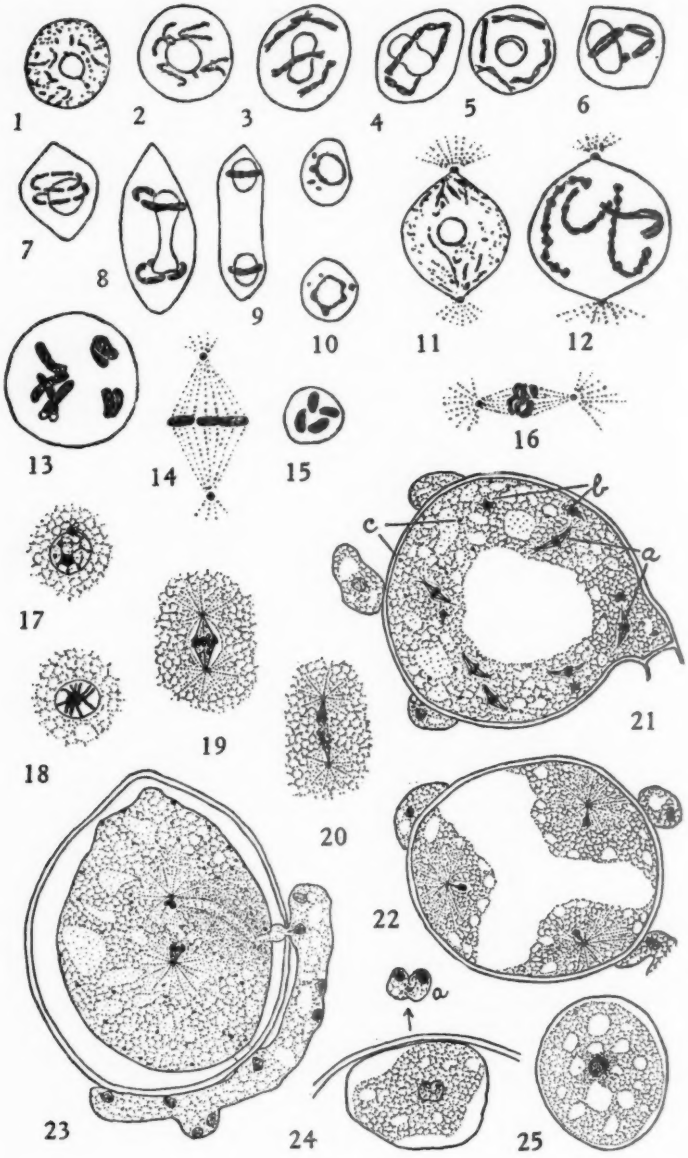
PLATE VI

FIG. 59. Diagram of nuclear history in the life cycle of a heterothallic ascomycete.

FIG. 60. Diagram of nuclear history in the life cycle of a heterothallic basidiomycete. (Both figures, by permission, from Fundamentals of Cytology, by L. W. Sharp, 1943. McGraw-Hill Book Company, Inc.)

PLATE VII

FIGS. 61–74. Karyogamy and meiosis in *Coleosporium vernoniae*. 61, binucleate basal cell and probasidium with a pair of prefusion nuclei; 62, early stage in karyogamy; 63, 64, zygotene, showing synapsing chromosomes; 65, synizesis; 66, zygotene chromosomes; 67, phase of chromosomal elongation, with nucleolus degenerating; 68, early diplotene; 69, late diplotene; 70, diakinesis; 71, late metaphase spindle with polar radiations; 72, anaphase; 73, late telophase, with astral rays recurving and assisting in the delimitation of the cross wall; 74, second meiotic division, with recurving astral rays. × 1410. (After Olive, 1949)



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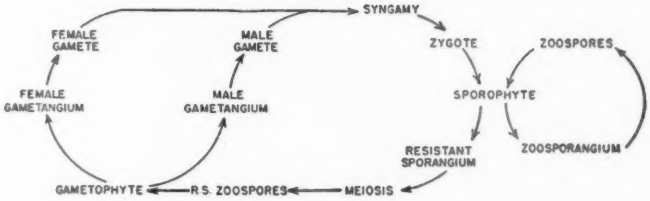
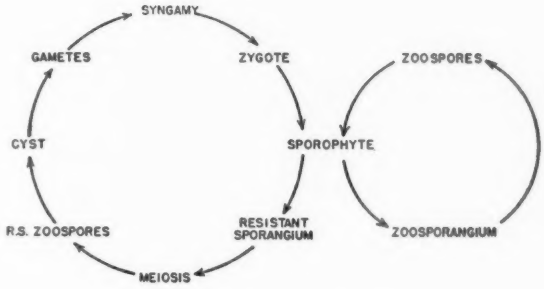
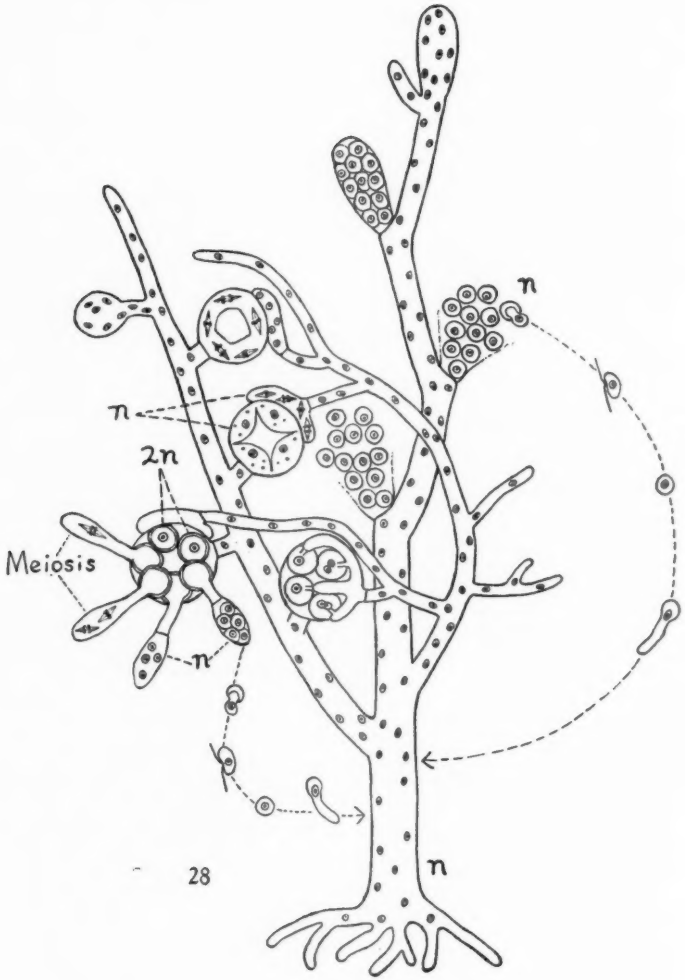


26 EUALLOMYCES LIFE CYCLE



27 CYSTOGENES LIFE CYCLE

PLATE II



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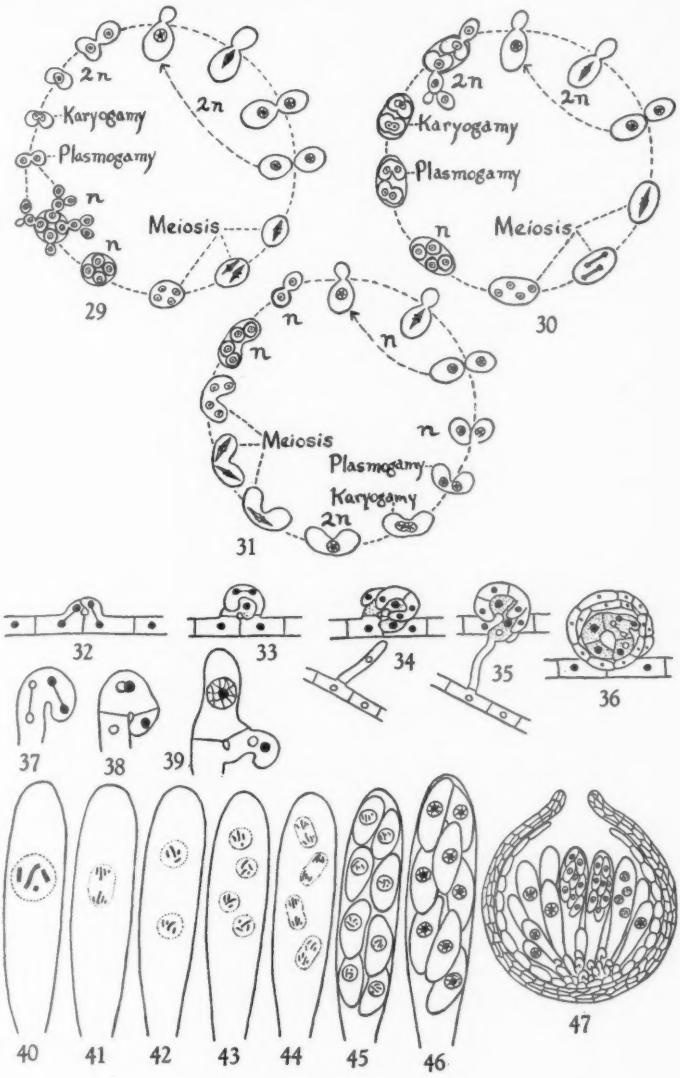
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Meiosis
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Karyogamy
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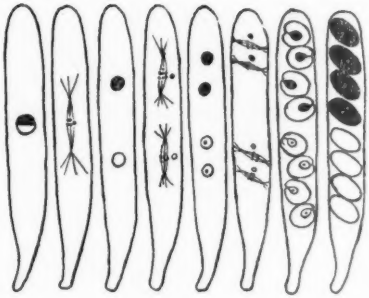
PLATE IV



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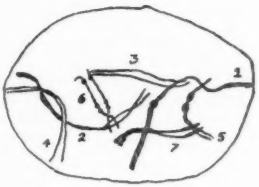

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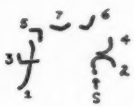

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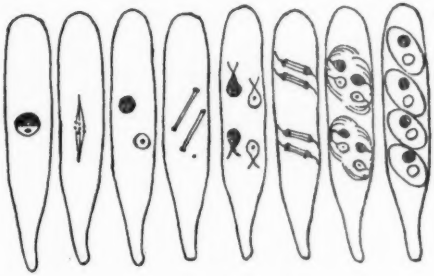

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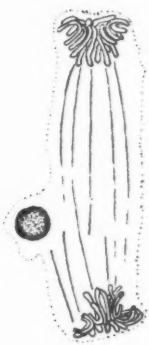

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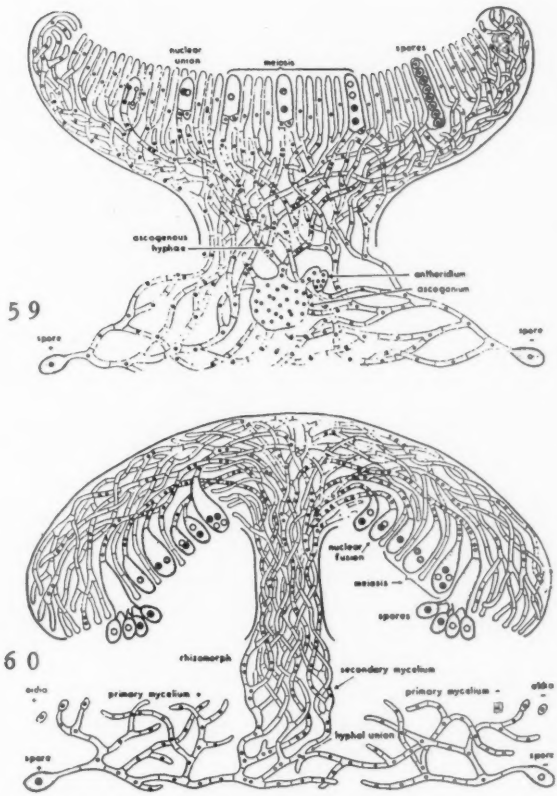
PLATE V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spores
ascogenous hyphae
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60
nuclear fusion
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primary mycelium
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PLATE VI

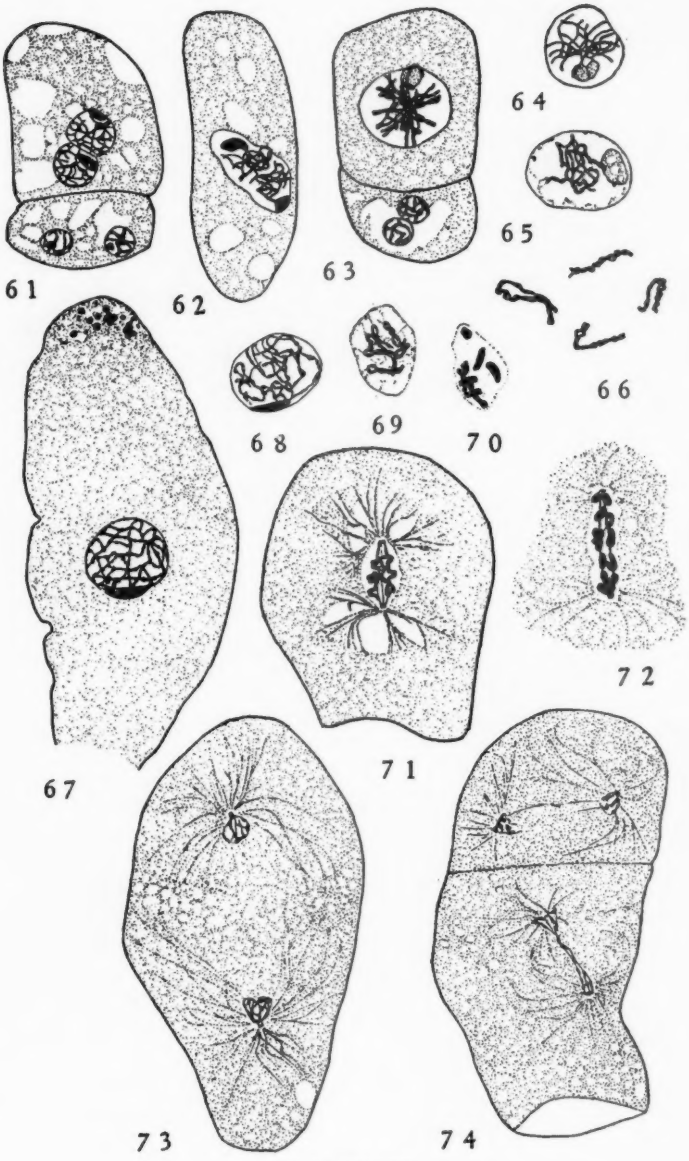
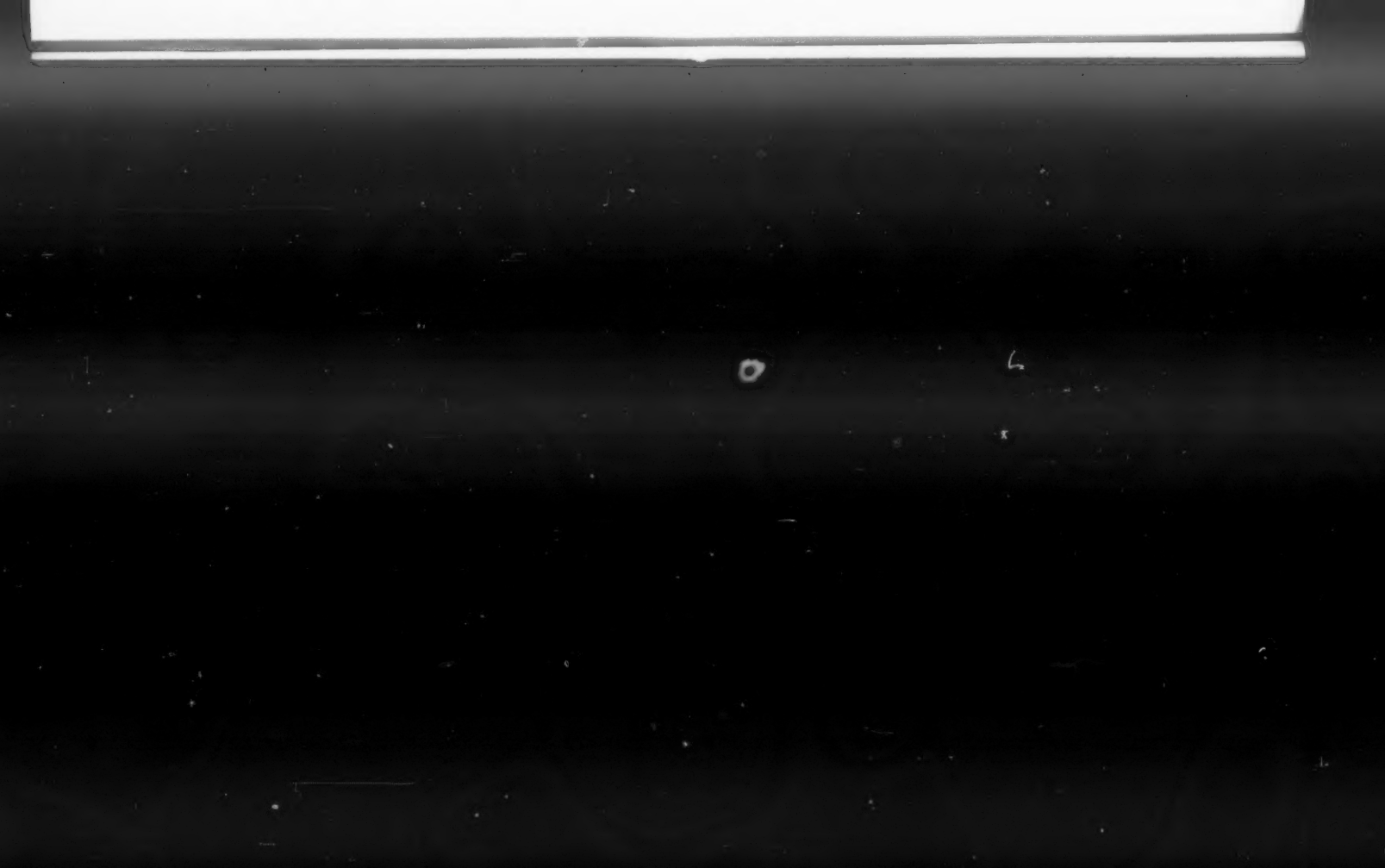


PLATE VII



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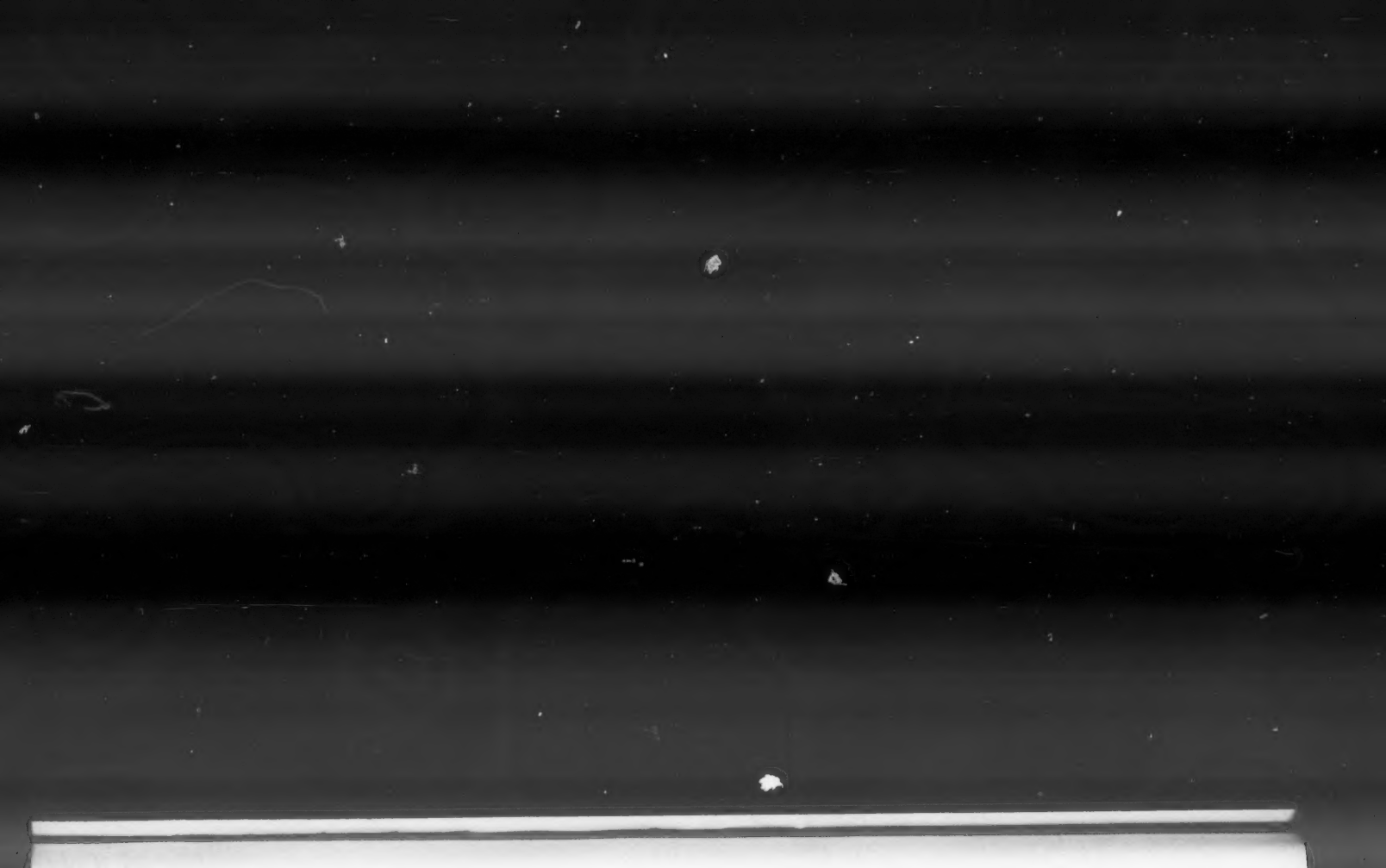
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Algae: Planktonic groups	R. ROSS *British Museum, London*
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Bryophytes	WILLIAM CAMPBELL STEERE *Stanford University*
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